Scientific Edition

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

JUSTIN L. POWERS, EDITOR, WASHINGTON, D. C.

VOLUME XXXIII *MARCH, 1944* NUMBER 3
CONSECUTIVE No. 5

The Present Status of Analeptics*,†

By Lloyd W. Hazleton

By broad general definition an analeptic is any agent which restores health, a definition derived from its Greek origin. Obviously a discussion of analeptics on this basis would constitute a treatise on therapeutics in its broadest concept. Somewhat more specifically the term analeptic has been applied to drugs with stimulant action, particularly on the central nervous system. This is the sense in which Clark (1) uses the term. The term is further restricted by Goodman and Gilman (2) to that group of drugs which are used to lighten narcosis. A definition such as the latter is predicated upon a precise understanding of the implications of narcosis, whereas no such agreement exists. It does, however introduce a definition based on the usage of the drugs, *i. e.*, a therapeutic classification in contrast to the pharmacological.

In view of the above it may be well to question whether the classification of certain drugs as "analeptics" serves any useful purpose. From a pharmacological standpoint the answer is no, because all of the drugs which are used as analeptics can be more accurately classified as central nervous system stimulants or as irritants. It should be remembered, however, that all such drugs are not analeptics and that segregating certain of them into this therapeutic classification serves a useful purpose.

To delineate the scope of the present discussion the term "analeptic" as used hereafter applies only to those drugs which are used to stimulate the vital medullary centers when these centers are in a depressed state. Such a definition carefully avoids the use of the term narcosis, since Hazleton and Koppanyi (3) and others have shown that the analeptic drugs are to be avoided in the treatment of poisoning by morphine or its related compounds, yet these drugs are generally considered typical of the narcotic class. While the principal action is on the medullary centers it may be direct or by reflex stimulation, and is usually accompanied by other actions. Perhaps the most important limitation to the analeptic class is the requirement that the drug be capable of stimulating the vital centers when they are under severe depression by other agents or causes. With the scope of the subject thus delineated, it is possible to discuss the mechanism involved in analeptic action and the individual agents which are either analeptics in the true sense or are used as such on an empirical basis.

* Received January 5, 1944, from The George Washington University, School of Pharmacy, Washington, D. C.

† This is the sixth of a series of review articles. Part of the cost of publication of this series of review articles is paid from the treasury of the National Conference on Pharmaceutical Research.

MECHANISM OF ANALEPTIC ACTION

In its normal state an animal organism is in equilibrium with its environment. Such vital factors as temperature, blood pressure and gaseous exchange are regulated by intricate and interdependent reflexes. Almost without exception a *tendency* away from equilibrium of any of these factors results in compensating reflexes which act to maintain equilibrium. If drugs capable of stimulating the central nervous system of animals (including humans) are administered, their action on the vital medullary centers is masked by compensating reflexes unless the dosage is extreme. The action on the spinal cord or cortex may at the same time be manifest, since it is possible to cause convulsions with strychnine or excitement with caffeine preceded by only a slight or temporary effect on respiration. If the normal functioning of the compensatory reflexes is interfered with by the action of depressant drugs on the medullary centers the result is loss of equilibrium for the duration of such action. Since respiration is the most important consideration in the action of depressant drugs and the use of analeptics it may be well to consider this in a typical example.

If a toxic dose of barbiturate is given to an animal there ensues a series of events following a definite pattern: drowsiness, muscular incoördination, stupor and coma. In this condition equilibrium no longer exists owing to an increase in the threshold of response of the respiratory center, and there is an increase in the carbon dioxide content of the blood with a corresponding decrease in the oxygen content. So long as the center continues to respond in some degree to stimulation by carbon dioxide or anoxia impaired respiration will continue and the animal will survive. During this period the blood pressure remains normal or elevated in an attempt to supply oxygen to the tissues. If the effect of the drug decreases due to its destruction or excretion the condition becomes reversible and the animal will recover without the intervention of analeptics. On the other hand, severe and prolonged anoxia causes a decrease in the functional tone of the circulatory mechanism, both central and peripheral. Seriously impaired respiration accompanied by circulatory failure and reduced temperature results in a condition of shock which, if not treated, usually terminates fatally. In order to interrupt this vicious circle it is necessary to select the most vulnerable point. The decreased temperature is a result of reduced muscular activity and vasodilation and is not important as a point of attack. Circulatory failure, although dangerous, is a result of anoxia; attempts to restore peripheral blood pressure would be treating the effect, not the cause, and such measures are usually ineffective in shock. Since the primary cause of the condition is respiratory depression, it is logical that restoration of adequate respiration should be the immediate objective of therapeutic measures, and this is the primary function of analeptic drugs. Stimulation of the circulatory and higher centers is an important auxiliary action, and careful attention should be given to the prevention of shock and respiratory complications.

The administration of an analeptic to a severely depressed animal is followed by a series of events which vary according to the nature of the agent. The common requirement of all is that they be able to restore the failing respiration toward normal. In general this can be accomplished only by a direct action on the respiratory center, in opposition to the depressant drug. Reflex stimulation of the center, such as cold water on a fainted person, is common practice but is ineffective against the severe depression induced by a drug. The nature of the analeptic determines whether there are other evidences of central nervous system stimulation. Thus metrazol induces a marked arousal of the animal due to cortical stimulation, whereas picrotoxin in equally life-saving doses may not arouse the animal. Under similar conditions strychnine may cause typical spinal convulsions without life-saving effect if the dose of the depressant is several times the lethal dose. In all cases care must be exercised to avoid excessive doses of the analeptic since the normal pharmacology of these agents is such that they cause depression or fatigue of the respiratory center following repeated convulsions. If this depressant action is added to the preëxisting depression, the outcome is almost surely fatal (4, 5, 6).

METHODS OF INVESTIGATION

The literature on analeptics is voluminous and confusing and the discrepancies between clinical and laboratory results are often difficult to rationalize. As a result any evaluation of the relative merits of an analeptic agent must be based on a careful study of the conditions under which the investigation is conducted. It is almost trite to point out that, as discussed above, analeptics are not essential for recovery from other than lethal doses of depressant drugs, although hastened recovery may be clinically desirable and therapeutically ra-

tional. The mere fact of clinical recovery from an overdose of hypnotic following administration of an analeptic is not, *per se*, to be taken as evidence of the efficacy of the analeptic. Many variable factors enter into clinical evaluation, some of the most important being: inadequate knowledge of the lethal dose for humans and of the total amount of drug administered, difficulty in determining the particular drug involved and in establishing time elapsed before treatment, and hesitancy in using large, adequate doses of the analeptic.

Laboratory experimentation avoids most of these variables, but the results are not directly applicable to the clinic. This disadvantage is largely overcome by the marked similarity of action in animals and humans, the principal difference being in dosage. The use of large numbers of animals and carefully controlled conditions make the laboratory approach more reliable as a basis for comparing the relative life-saving properties of various analeptics. The scope of the present discussion does not permit an attempt to describe all of the techniques used in the laboratory, but a few of the underlying principles may be outlined. The first step is observation of the effects following administration to normal animals. These may include effects on the vital centers, excitement, tremors or twitches, convulsions, and death. From these preliminary experiments on various species and with different routes of administration a general dosage range may be determined, after which the convulsive and lethal doses may be established. The dose sought for the latter two is usually that one which will produce the desired effect in about fifty per cent of the animals tested. This dose is then expressed as the CD_{50} or LD_{50} for the convulsive and lethal doses, respectively. The figure is accurately established only after large numbers of experiments are subjected to statistical evaluation. It may also be desirable to determine the smallest dose which will exhibit an effect and this is called the minimal or the therapeutic dose. The figure obtained by dividing the lethal dose by the convulsive (or the smaller therapeutic) dose is termed the therapeutic ratio or index. This value serves, within certain limitations, as an evaluation of the relative safety of different drugs. The therapeutic index loses much of its significance in the case of analeptics because the mutual antagonism between depressant and stimulant drugs permits administration of much more than the lethal dose of the latter.

Having established these basic values the investigators are interested in the potency of the analeptic action. This usually involves at least two groups of experiments. The first group seeks an evaluation of the action in anesthetized animals, under standard conditions which are familiar to the investigator. Much of this type of work makes use of some member of the barbiturate family to induce a uniform degree of anesthesia, although other anesthetics of the fixed or volatile type are frequently used for specific information. After induction of anesthesia in a number of animals the analeptic is administered and the recovery time of the animals is compared to the recovery time of the untreated controls. The second group of experiments is directed toward a determination of the life-saving properties of the drug following administration of surely lethal doses of the anesthetic. This, obviously, is the crucial type of experiment in the final evaluation of the merits of the drug. Unless an analeptic agent is capable of stimulating the vital centers during extreme depression it is valueless in emergencies, even though it does lighten the anesthesia induced by nonlethal doses.

If the agent shows promise of usefulness as an analeptic in the above experiments there is much more information to be compiled, such as duration of action, rapidity of action, rate of absorption, rate of essential elimination and its ultimate fate in the body. Only when this and other data are available from well-conducted experiments can the results be correlated with such clinical reports as are available, and the rational place of the analeptic in the therapeutic armamentarium be established.

In the foregoing discussion of analeptics in general, an attempt has been made to outline only the most fundamental considerations and to avoid intimate detail and controversial subjects. It is hoped that it will furnish sufficient background for the interested reader and that specialists in the field will find only errors of omission, not of commission.

ANALEPTIC AGENTS

The following discussions are confined to the analeptic action of the drugs, with reference to their other actions only as these bear on their use as analeptics. Citation of specific papers is made only as a guide to further reading and the inclusion or omission of papers is not necessarily indicative of their relative merits. Since all these agents are central nervous system stimulants, no further classification is made, except in a general way according to status in therapeutic use.

Picrotoxin.—Picrotoxin is a nonalkaloidal,

nonglycosidal principle from cocculus. Picrotoxin and Picrotoxin Injection were additions to the United States Pharmacopœia, twelfth revision. It should not be assumed that picrotoxin is a new drug, however, for it was discovered early in the 19th century and by the turn of the century much of its pharmacology was known. For a review of the early history the reader should refer to the papers by Maloney, *et al.* (7), and Koppanyi, *et al.* (8); suffice it here to say that by 1900 the analeptic action of picrotoxin against the then known depressants such as chloral hydrate, paraldehyde, sodium bromide and morphine had been investigated. The reports are at considerable variance with each other and are of historical interest only in view of the more extensive investigations which have been made since the very significant work of Maloney, Fitch and Tatum in 1931 (7). All of the work, both experimental and clinical, leading to official recognition of picrotoxin has been done since the publication of this paper describing the analeptic value of picrotoxin in barbiturate poisoning.

The medullary stimulant action of picrotoxin is so closely associated with the analeptic properties that very few papers dealing specifically with this action have been published, although the majority of authors agree that this is the primary action. Marshall, *et al.* (9), point out that since picrotoxin has very little effect on the respiration of intact animals but a powerful action on the depressed respiration, the action must not be pure stimulation of the medulla, but a decrease in the narcosis which makes the animal more normal. Regardless of the intimate mechanism of action, these authors attribute the powerful analeptic action to medullary effects and show that picrotoxin can initiate respiration which has been stopped by various surgical and pharmacological means. In addition to respiratory stimulation the medullary action of picrotoxin increases the blood pressure by stimulation of the vasomotor center. This action no doubt enhances the analeptic potency of picrotoxin, but, as previously discussed, is secondary in importance. Other evidences of medullary stimulation are salivation, nausea and vomiting in the normal animal.

Although the principal action of picrotoxin is medullary stimulation, this is accompanied by some degree of cortical and spinal stimulation. The evidence of cortical stimulation is reviewed and supplemented by Koppanyi, *et al.* (8). These authors found that after depression of the cortex by minimum anesthetic doses of barbiturates, leading to loss of superficial and postural reflexes, the administration of suitable doses of picrotoxin would produce an awakening effect as evidenced by return of these reflexes. If the dose of barbiturate exceeded twice this minimum anesthetic dose no awakening could be induced, although the life of the animals could be saved after approximately four times the lethal dose. Similar results by other investigators emphasize the fact that awakening or other evidences of cortical stimulation are not essential to the primary action of medullary stimulation. It has also been shown (6) that large doses of picrotoxin, particularly if there are convulsions, cause a depression of certain cortical functions. This is true even in the presence of barbiturate anesthesia, in which case the return of these cortical reflexes may be delayed beyond the duration of the anesthesia. Certain features of the spinal action of picrotoxin have been described (3), which indicate that premedication with morphine or dilaudid sensitizes the convulsive action of picrotoxin without simultaneous stimulation of the depressed respiration in rabbits. Since there was no evidence of medullary stimulation, and the convulsions were of the spinal type, the authors conclude that the observed synergism was between the spinal action of the two drugs.

In general these accessory actions do not alter the character of the almost pure medullary stimulant action exerted by picrotoxin in the presence of severe depression. Doses of depressants sufficiently large to merit intervention with analeptics will antagonize the cortical effects of picrotoxin, and large doses of depressants other than morphine so depress the spinal cord that the spinal stimulant action is masked. There remains the danger, however, that excessive analeptic doses, which lead to convulsions, will result in a prolonged period of cortical depression.

Following the administration of picrotoxin there is a characteristic delay before the onset of action. After intravenous administration there is a latent period of twelve to seventeen minutes before the onset of convulsions (6); after intraperitoneal or intramuscular administration this period is even longer. The experiments of Duff and Dille (10) would indicate that perhaps this time is required for the drug to become distributed into the tissues since the blood concentration drops rapidly after intravenous administration, leveling off after about twenty minutes. During this time the concentration in liver and muscle tissue increases to the maximum, after which time all the values decrease until at the end

of about two hours no more than traces of picrotoxin remain in the blood, liver or muscles. In another study essentially in agreement with the above, Dille (11) has shown that picrotoxin is rapidly detoxified, although traces may be found in the urine of rabbits for as long as eighteen hours.

The striking efficiency of picrotoxin as an analeptic in the laboratory led to its clinical trial several years before much of the pharmacology discussed above had been elucidated. The first clinical use of picrotoxin in acute barbiturate poisoning was reported by Arnett (12), but the degree of poisoning and the very small doses of picrotoxin administered were inconclusive as an index of analeptic potency. The first use against lethal doses of barbiturate is reported in two cases by Murphy, *et al.*, in 1937 (13). By 1939 the number of clinical reports were rapidly increasing, for early in that year Dille (14) tabulated a total of fifteen cases and somewhat later Reifenstein (15) reviewed thirty-six cases and added two new ones. At about this time the Council on Pharmacy and Chemistry of the American Medical Association (16) reviewed the status of picrotoxin and recommended its use in cases where the results might be carefully studied. It also advised caution and pointed out that the pharmacology of large doses was still unknown.

A partial answer to this latter problem was supplied by the experimental work cited above and by Richards and Menaker (17). From a review of the literature and reports on eleven new cases these authors could find no evidence of organic injury even after very large doses. The striking feature of clinical reports on the use of picrotoxin in barbiturate poisoning is the unanimity of opinion on its effectiveness.

The status of picrotoxin as an analeptic against depressants other than barbiturates is rather unsettled. Marshall, *et al.* (9), conclude that picrotoxin is effective in overdosage with chlorobutanol, paraldehyde or avertin, less effective against urethane and useless against ethyl alcohol. These findings substantiate those of Barlow (24), who found it effective against avertin and chloral hydrate. The contraindication of picrotoxin in morphine poisoning is discussed above.

In clinical practice there is no fixed dosage for picrotoxin since the dose depends upon the degree of depression present. The U. S. P. dose of 2 mg. or more, depending on the severity of the barbiturate poisoning, takes cognizance of this fundamental principle. Bleckwenn and Masten (18) suggest intravenous administration at the rate of 1 mg. per minute until reflexes return. This method appears to neglect two factors since it does not supply the desirable large initial dose and does not allow for the latent period of action which may result in excessive stimulation after the administration has been stopped. Murphy, *et al.* (13), recommend an initial dose of 5 mg. intravenously, to be repeated in twenty minutes if no signs of stimulation ensue. Subsequent doses of 10 mg. should then be given at twenty-minute intervals until signs of stimulation appear, the first signs being muscular twitches. Smaller doses of from 3 to 5 mg. may then be given to maintain the patient in satisfactory condition. The dosage suggested by Fantus and Richards (19) is comparable, being 6 to 12 mg. at intervals of at least fifteen minutes. In a report on twenty cases Anderson (20) found picrotoxin to be the agent of choice in barbiturate poisoning. He recommends its prompt intravenous administration in all cases where the reflexes are abolished, to be repeated until corneal reflexes reappear and the patient responds to painful stimuli. To maintain the patient, doses of 3 to 6 mg. per hour may be given. In all these programs it should be noted that the end point is return of superficial reflexes rather than arousal or other evidences of cortical action.

Since no definite dose can be stated for picrotoxin it may be well to review the fundamental factors which influence the posology of this drug. They are rather definite and well established and when followed should provide a satisfactory margin of safety for the clinical use of picrotoxin as an analeptic.

(*a*) The type of depressant drug being treated should be known. If morphine or its compounds can be ruled out, picrotoxin may be given, since there is little evidence that it is contraindicated in the treatment of other depressants. It must be remembered, however, that adequate evidence of its analeptic potency exists only in the case of barbiturates. While chemical tests exist (21) for the detection of barbiturates in the blood and urine, and the method has recently been refined (22), the time involved frequently precludes their use before treatment is begun. In such cases diagnosis must be predicated on symptoms and case history.

(*b*) Picrotoxin and the barbiturates are mutually antagonistic; therefore the total amount of picrotoxin administered will be much in excess of the total that could be tolerated by a normal individual. It is this factor which governs the size of the individual dose to be given; a patient in coma will tolerate up to 10 mg. of picrotoxin with-

out overstimulation, whereas a larger dose may prove to be excessive. If this initial dose does not evoke signs of stimulation it is usually safe to repeat it, but once signs of stimulation appear, the dose must be reduced.

(*c*) Arousal of the patient is not essential to a favorable prognosis, and this should not be sought in picrotoxin therapy, although it may occur if the depression is not severe. The desired end point is improvement in muscular tone, return of superficial reflexes and satisfactory respiration and blood pressure. This condition may be accompanied by slight twitches of the facial muscles.

(*d*) Convulsions should be avoided since these lead to depression of the respiratory center and an unfavorable prognosis. In the laboratory such overdosage with picrotoxin can be treated by additional barbiturate, but this is obviously not desirable clinical procedure if it can be avoided.

(*e*) Administration of repeated doses should be spaced to take cognizance of the latent period inherent in the action of picrotoxin even after intravenous administration. The minimum time to be allowed for full development of the effect should be fifteen minutes. If the exigency of the case permits, a period of twenty minutes would be safer.

(*f*) Under no circumstances should picrotoxin therapy preclude the employment of other therapeutic measures designed to improve the condition of the patient and to avoid shock and respiratory complications.

Metrazol.—Metrazol is pentamethylenetetrazol and was introduced in Europe under the name of cardiazole. It is a white crystalline powder, freely soluble in water and very stable under normal conditions. While metrazol is sometimes referred to as a camphor substitute there is little basis for such a classification.

Metrazol is a comparatively recent addition to therapeutics, its synthesis being based on a reaction described by Schmidt in 1924 (23). The principal pharmacological action of metrazol is stimulation of the medullary centers. This is accompanied by stimulation of the spinal, cortical and subcortical areas. For detailed information on the pharmacology of metrazol, the extensive review of Heffter (23) is suggested.

Large doses of metrazol produce convulsions of the predominantly clonic type in both mammals and frogs. These convulsions are antagonized by the barbiturates and other depressants. That this is a mutual antagonism is adequately demonstrated by the reports of Koppanyi, *et al.* (8), and by Barlow (5, 24). These authors found that animals could be immediately awakened from anesthetic doses of barbiturates by the injection of metrazol, and conversely, that barbiturates prevented convulsions following large doses of metrazol. Barlow also found metrazol to be effective against chloral hydrate and avertin, while McCrea and Taylor (25) found that it restored respiration in dogs after the respiration had been stopped by intravenous ethyl alcohol. In common with the authors previously quoted, Koppanyi (8) and Marshall (9) found that while metrazol was effective against severe barbiturate depression, its extreme range of potency is inferior to that of picrotoxin.

The onset of action of metrazol is extremely rapid, usually being manifest less than one minute following intravenous injection. As is usual with rapidly acting drugs, the duration of action is relatively brief and the patient or animal may relapse following arousal with metrazol if the depressant is long acting. This may be overcome by repeated injections or by following the intravenous injection with intramuscular injections which are slower but longer acting.

After reaching the blood stream metrazol is universally distributed and rapidly eliminated. Tatum and Kozelka (26) administered large doses of metrazol to rabbits under paraldehyde anesthesia and found that the rate of destruction was in direct proportion to the concentration of drug in the tissues. They observed that the kidney has little effect on the destruction, while the liver plays an important role. These observations were based on chemical assay of the drug in blood and tissues. Using a pharmacological rather than a chemical approach to the same problem, Dille and Seeberg (27) found that in cats the drug is destroyed rather than excreted, and that the liver plays an important part in the destruction.

The dose of metrazol is subject to the same factors that govern the dose of picrotoxin, with but few exceptions. Since metrazol acts rapidly and briefly the dose may be repeated at more frequent intervals without the dangers of delayed action or overdosage. Experimental evidence that inadequate dosage with metrazol may actually prolong barbiturate anesthesia (6) may explain the clinical failures occasionally reported. A typical dosage scheme is reported by Walker and Teague (28) who gave 5 cc. of the 10% solution intravenously and followed this with 2 cc. every two hours. The first dose restored body movements and this condition was maintained by the

subsequent doses until recovery was complete. The arousal or awakening effect of metrazol is quite prominent and it is probable that any dose which does not restore some evidence of cortical activity is inadequate. This prominent action and the ease of control make metrazol the drug of choice in mild depression. The incidence of twitches or even mild convulsions is not a serious complication, but calls for a reduction in the rate of administration of the drug. Since the convulsive actions of metrazol and morphine are synergistic (3), the use of metrazol in severe morphine poisoning is a dangerous procedure.

Strychnine.—Strychnine is an alkaloid obtained chiefly from nux vomica. The alkaloid and several salts are official in the U. S. P. and N. F. The first pharmacological work on strychnine was done by Magendie in the early 19th century. In his classical experiment with the ligated leg of the frog Magendie demonstrated that the stimulant action of strychnine was central rather than peripheral. The action of strychnine is most prominent on the spinal cord and strychnine is usually referred to as a spinal stimulant. Dusser de Barenne (29) has shown that both the motor and sensory cells in the reflex arc must be acted upon by strychnine, while Heinbecker and Bartley (30) have shown that strychnine exerts a combination of effects by a lowering of the threshold of excitability, prevention of loss of excitability following continuous stimulation, and prolongation of the period of hyperexcitability following nerve stimulation. Even though excessive doses of strychnine result in prolonged and violent tonic convulsions, the drug has no direct stimulant action but acts by sensitizing the nervous system to all types of external stimuli so that these become highly exaggerated and incoördinated.

In addition to the prominent spinal action strychnine exhibits some action on the medulla, and to a lesser extent on the cortex. It appears probable that these actions are also sensitizing rather than direct, and would invariably be accompanied by spinal action unless the cord was specifically depressed. The stimulant phase of strychnine action is followed by a depressant phase and death from strychnine is due to respiratory failure (32). Small doses do not exhibit respiratory or circulatory effects since these would be compensated, but Henderson (31) believes that increased tonus in the skeletal muscles may result in improved circulation and respiration.

As might be expected from the above description of strychnine action, the antagonism between strychnine and the central nervous system depressants tends to be unilateral rather than mutual. Very large doses of strychnine are antagonized by depressants, whereas strychnine is only moderately antagonistic to the depressants. Lack of mutual antagonism is due to the fact that the depressants cause fatal depression of the medullary centers and the medullary stimulant action of strychnine is not sufficiently prominent to overcome the depression, even in doses which stimulate the depressed cord to convulsions. On the other hand, large doses of strychnine may be antagonized by the spinal action of the depressants without excessive depression of the medulla. This property of the depressant drugs is predicated upon their classic sequence of action, namely, depression of the cortex, spine and medulla in that order. For further details on this interaction the reader is referred to the work of Gold and Travell (32*a*) on ethyl alcohol and strychnine and to Maloney (33) on barbiturates and strychnine.

A critical review of the experimental and clinical reports on strychnine as an analeptic raises very serious doubt as to whether this drug has any place in this category. Certainly its efficacy is doubtful in the treatment of severe barbiturate poisoning (7, 19) and most clinical reports are those in which medication consists of two or more analeptics. Examples of such clinical reports are the use of ephedrine and strychnine (2) and of strychnine, caffeine, coramine and atropine hypodermically (34). The contraindication of analeptics in severe morphine toxicity has been discussed, and this would seem particularly important in the case of strychnine with its prominent spinal action.

After intravenous administration of strychnine the onset of action is intermediate between metrazol and picrotoxin, the latent period for a convulsive dose being three to four minutes (35). Weiss and Hatcher (36) found that the liver was responsible for the destruction of a large portion of the strychnine in the body, while the kidneys excreted only that which the liver failed to destroy. The essential elimination of strychnine under various conditions has been reported by Hazleton and Fortunato (35), who found the rate in rats to be approximately one-half convulsive dose per hour, and somewhat slower in rabbits.

The clinical dosage is in general governed according to the degree of depression. In cases of severe depression doses of 10 mg. may be given intravenously or subcutaneously and repeated at hourly intervals

until signs of stimulation appear. These signs are likely to be spinal in origin and convulsions may occur from overdosage without significant medullary stimulation.

Coramine.—Coramine is the proprietary name for the diethylamide of nicotinic acid. The nonproprietary name of nikethamide has been assigned to this compound by the Council on Pharmacy and Chemistry of the American Medical Association, and it is now listed under that name in "New and Nonofficial Remedies" (37).

The principal pharmacological action of coramine is stimulation of the central nervous system. In therapeutic doses there is little effect on the respiration or circulation of normal animals, but large doses cause convulsions distinctly clonic in type. Respiration which has been depressed is stimulated by coramine, but there is little evidence of its effectiveness against more than lethal doses (7, 9, 24), although numerous clinical reports are favorable (38, 39). It has been shown that morphine synergizes with the convulsant action of coramine (3), and coramine might therefore be contraindicated in severe morphine poisoning.

Lack of life-saving properties against very large doses of depressants is probably accounted for by the type of stimulation produced. Coramine causes excitement followed by coördinated clonic convulsions suggestive of cortical stimulation. Schulte, *et al.* (40), make use of this characteristic type of stimulation to differentiate coramine, caffeine and cocaine from picrotoxin and metrazol which are more prominently stimulant to the medulla.

Coramine is available in 25% aqueous solution, the initial clinical dose being 3 cc. by any route. If used as an analeptic the dose would be markedly increased and subject to variation according to the degree of depression.

Caffeine.—Caffeine has several important pharmacological actions, among them being stimulation of the central nervous system. This central action is principally cortical (40) and therefore of limited importance as an analeptic in severe depression. In this respect it has been found definitely inferior to picrotoxin (4) and to picrotoxin, metrazol, ephedrine, coramine and strychnine (24). The cortical action of caffeine undoubtedly has some value in the termination of moderate depression and relieving the mental aftereffects.

Sympathomimetic Amines.—This group of drugs is comprised of the natural and synthetic derivatives of epinephrine. For an extensive review of the chemistry and its relation to pharmacological activity the reader is referred to the report of Hartung (41). A comparison of the analeptic potency of a series of the amines has been made by Tainter, *et al.* (42).

In general these compounds differ pharmacologically from epinephrine in that they are effective orally, have a longer duration of action and are stimulant to the central nervous system. Some of the more commonly known sympathomimetic amines are ephedrine, benzedrine, neo-synephrine, kephrine and propadrine. Only ephedrine and benzedrine are important as analeptics and both of these have received experimental and clinical trials.

Theoretically these compounds might have advantages as analeptics since they have a prominent action on the cardiovascular system, causing a rise in blood pressure in addition to the respiratory stimulation. There is little question but what these agents are of value in lightening anesthesia and hastening recovery from nontoxic doses of the depressants. When depression is severe, however, the medullary stimulant action is inadequate and in shock it has been repeatedly demonstrated that these compounds are ineffective in restoring the peripheral blood pressure.

The effect of benzedrine on the narcosis produced by various depressants has been studied by numerous investigators, typical examples being anytal (43), avertin (44), alcohol (45) and morphine (46). To date there is little evidence favoring the choice of the sympathomimetic amines over picrotoxin and metrazol where life-saving properties are the criterion.

Carbon Dioxide.—Carbon dioxide is the natural stimulant of respiration. In many cases of respiratory insufficiency it is the agent of choice because of its efficiency and safety. In cases of severe respiratory depression due to certain drugs there is an excess of carbon dioxide to which the center is unresponsive, therefore the administration of carbon dioxide is of no value. Most of the volatile anesthetics are respiratory stimulants in small doses and may cause acapnia from overbreathing. In such cases carbon dioxide is valuable as a regulating agent, but such use cannot be considered as analeptic since removal of the anesthetic would be followed by recovery after a period of apnea (47). Excessive dosage with the volatile agents induces depression of the center and carbon dioxide becomes ineffective. Henderson (31), in a review of respiratory stimulants, discusses these factors in more detail.

Miscellaneous.—From time to time the

literature contains reports of investigations on the analeptic potency of various drugs. Some of those most frequently encountered are camphor, cocaine, lobeline, coriamyrtin, tutin, thyroxin and ethyl alcohol. As a group these agents appear to be definitely inferior to those described above and at the present time the favorable evidence does not seem to justify individual discussion. Additional research on some of these compounds is undoubtedly desirable, but it seems most likely that future advances in the field of analeptics will come from the vast unknown of synthetic drugs.

REFERENCES

(1) Clark, A. J., "Applied Pharmacology," The Blakiston Company, Philadelphia, 1940.

(2) Goodman, L., and Gilman, A., "The Pharmacological Basis of Therapeutics," The Macmillan Company, New York, 1941.

(3) Hazleton, L. W., and Koppanyi, T., *Anesthesiology*, 2 (1941), 427.

(4) Maloney, A. H., *Quart. J. Exptl. Physiol.*, 25 (1935), 155.

(5) Barlow, O. W., *J. Lab. Clin. Med.*, 23 (1938), 601.

(6) Dille, J. M., and Hazleton, L. W., *J. Pharmacol.*, 67 (1939), 276.

(7) Maloney, A. H., Fitch, R. H., and Tatum, A. L., *Ibid.*, 41 (1931), 465.

(8) Koppanyi, T., Linegar, C. R., and Dille, J. M., *Ibid.*, 58 (1936), 199.

(9) Marshall, E. K., Jr., Walzl, E. M., and Le-Messurier, D. H., *Ibid.*, 60 (1937), 472.

(10) Duff, D. M., and Dille, J. M., *Ibid.*, 67 (1939), 353.

(11) Dille, J. M., *Ibid.*, 64 (1938), 319.

(12) Arnett, J. H., *J. Am. Med. Assoc.*, 100 (1933), 1593.

(13) Murphy, W. S., Connerty, H. V., Connolly, A. J., and Koppanyi, T., *J. Lab. Clin. Med.*, 22 (1937), 350.

(14) Dille, J. M., *Northwest Med.*, 38 (1939), 80.

(15) Reifenstein, E. C., Jr., Reifenstein, E. C., Sr., Mignault, J. W., and Raffaele, A. J., *Ann. Internal Med.*, 13 (1939), 1013.

(16) Council on Pharmacy and Chemistry, American Medical Association, *J. Am. Med. Assoc.*, 112 (1939), 431.

(17) Richards, R. K., and Menaker, J. G., *Anesthesiology*, 3 (1942), 37.

(18) Bleckwenn, W. J., and Masten, M. G., *J. Am. Med. Assoc.*, 111 (1938), 504.

(19) Fantus, B., and Richards, R. K., *Ibid.*, 115 (1940), 527.

(20) Anderson, J. P., *Ann. Internal Med.*, 14 (1941), 2037.

(21) Koppanyi, T., Dille, J. M., Murphy, W. S., and Krop, S., THIS JOURNAL, 23 (1934), 1074.

(22) Green, M. W., Veitch, F. P., Koppanyi, T., *Ibid.*, 32 (1943), 309.

(23) Heffter, A., "Handbuch der Experimentellen Pharmakologie," J. Springer, Berlin, 5 (1937), 151.

(24) Barlow, O. W., *J. Pharmacol.*, 55 (1935), 1.

(25) McCrea, F. D., and Taylor, H. M., *Ibid.*, 68 (1940), 41.

(26) Tatum, H. J., and Kozelka, F. L., *Ibid.*, 72 (1941) 284.

(27) Dille, J. M., and Seeberg, V. P., *Pharm. Arch.*, 12 (1941), 9.

(28) Walker, R. H., and Teague, F. B., *Virginia M. Monthly*, 69 (1942), 92.

(29) Dusser de Barenne, J. G., *Physiol. Revs.*, 13 (1933), 325.

(30) Heinbecker, P., and Bartley, S. H., *J. Physiol.*, 125 (1939), 172.

(31) Henderson, Y., "The Pharmacopœia and the Physician," American Medical Association, Chicago, 1939.

(32) Travell, J., and Gold, H., *J. Pharmacol.*, 53 (1935), 169.

(32*a*) Gold, H., and Travell, J., *Ibid.*, 52 (1934), 30.

(33) Maloney, A. H., *Ibid.*, 49 (1933), 133.

(34) Purves-Stewart, J., and Willcox, W. H., *Lancet*, 1 (1934), 6.

(35) Hazleton, L. W., and Fortunato, F., THIS JOURNAL, 31 (1942), 289.

(36) Weiss, S., and Hatcher, R. A., *J. Pharmacol.*, 19 (1922), 419.

(37) "New and Nonofficial Remedies," American Medical Association, Chicago, 1943.

(38) Orford, T. J., *Can. Med. Assoc. J.*, 30 (1934), 65.

(39) Killian, H., *Anesthesia and Analgesia*, 14 (1935), 23.

(40) Schulte, J. W., Tainter, M. L., and Dille, J. M., *Proc. Soc. Exptl. Biol. Med.*, 42 (1939), 242.

(41) Hartung, W. H., *Chem. Revs.*, 9 (1931), 389.

(42) Tainter, M. L., Whitsell, L. J., and Dille, J. M., *J. Pharmacol.*, 67 (1939), 56.

(43) Reifenstein, E. C., Jr., and Davidoff, E., *Proc. Soc. Exptl. Biol. Med.*, 38 (1938), 181.

(44) Michelson, J., and Verlot, M., *Anesthesia and Analgesia*, 18 (1939), 59.

(45) Reifenstein, E. C., *J. Lab. Clin. Med.*, 27 (1941), 131.

(46) Handley, C. A., and Abrew, B. E., *Anesthesia and Analgesia*, 20 (1941), 212.

(47) Seevers, M. H., Stormont, R. T., and Hathaway, H. R., *J. Pharmacol.*, 68 (1940), 365.

Critical Graphical Methods for Calculating Isotonic Concentrations and Freezing Points of Aqueous Solutions*

By Frank M. Goyan, John M. Enright and Julian M. Wells

Although many methods for calculating the concentration of substances isotonic with body fluids have recently been reviewed by Husa and Rossi (1) there is still no adequate presentation of this subject from the physicochemical point of view. Such a presentation is needed because for most practical purposes an isotonic solution may be defined as one which has the same freezing point as that of a chosen standard. Husa and Rossi (1) bring out the important distinction between isotonic and isosmotic solutions but base their practical methods upon the freezing points of aqueous solutions of different substances. Other methods have been based entirely upon arbitrary approximations.

It is one of the purposes of this paper to present the subject in such a way that exact data may be used to arrive at the best approximate values for many compounds not yet studied cryoscopically. This may be accomplished without sacrificing the precision that may be attained in dealing with compounds for which exact data are available. While it is clearly understood that extreme accuracy is seldom needed in this work, some of the approximations heretofore used have deviated widely from experimentally determined values, while at the same time expressing results to four or five significant figures. This tendency to be entirely uncritical of the accuracy needed or attainable has unfortunately carried over to calculations based upon experimentally determined freezing points. One of our purposes in presenting this material is to attempt to clear up some of this misunderstanding and to sacrifice accuracy intelligently for the sake of rapidity in making the calculations. We have, however, provided algebraic and graphic methods for obtaining greater accuracy from good data whenever the data are available and the accuracy is needed.

This presentation is made possible by the fact that most chemical substances may be classified into groups on the basis of similar osmotic properties. While a study of the freezing points of solutions of different substances at the same per cent concentration fails to reveal this natural grouping, it becomes apparent when concentrations are expressed in molal units.

* Received July 7, 1943, from the College of Pharmacy, University of California, San Francisco, Calif.

THE MOLAL LOWERING OF THE FREEZING POINT

This comparison is facilitated by the use of a ratio obtained by dividing the experimentally determined freezing-point depression by the corresponding molal concentration of the solute. This ratio is referred to as the molal lowering of the freezing point or the molal freezing-point depression and is defined by Equation I.

$$L = \Delta t / C \qquad (I)$$

L = the molal lowering of the freezing point,
Δt = the depression of the freezing point produced by the solute (° C.),
C = the molal concentration of the solute (moles per 1000 Gm. of water for exact work).

Values for the molal lowering of the freezing point for many substances are tabulated in the International Critical Tables (2). This function is comparatively insensitive to changes in concentration and has the further advantage that salts of the same ionic type tend to exhibit the same molal lowering. These properties are demonstrated in Fig. 1 which shows eight solid curves representing average values of the molal freezing-point depression for substances of different types designated 1A, 1B, 2A, 2B, etc. The dotted curve shown in Fig. 1 represents the locus of points described by the algebraic equation obtained by substituting the freezing-point depression of blood serum (0.56° C.) for Δt in Equation I. Thus the intersection of any one of the solid curves with the dotted curve represents the concentration and molal freezing-point depression of substances of the specified type in solutions isotonic with blood serum.

CLASSIFICATION OF COMPOUNDS ACCORDING TO TYPE

The solid curves drawn in Fig. 1 represent average values. These curves are included primarily for the purpose of illustration. More exact curves for each substance represented in Table I were drawn, and the point of intersection with the dotted curve was read for each substance. The results were tabulated in Table I, showing the magnitude of the error likely to be introduced by using an average value for all substances of a given ionic type rather than exact values. It will be noted that substances that can be properly classified into the types listed seldom deviate from the corresponding average by more than 10%. Those which might be expected to deviate from this rule are salts of mercury, lead and cadmium because of their tendency to exist as weak electrolytes. Certain salts like sulfides and tetraborates likely to undergo extensive hydrolysis or dissociation are not predictable unless data on very similar substances are available. These abnormal electrolytes together with complex ones are collected together as members of type 5 or type M. These lists are useful in predicting the approximate behavior of similar compounds. It will be noted that values for potassium tetraborate differ from those of borax by less than 10%.

A systematic arrangement of numbers and letters has been adopted for the purpose of classifying nonelectrolytes, weak electrolytes and strong electro-

lytes. Type 1A is reserved for nonelectrolytes, 1B for weak electrolytes and types 2, 3 and 4 for simple strong electrolytes. The numbers 2, 3 and 4 refer to the number of particles formed by dissociation of one molecule in solution. Weak acids (all organic acids, phosphoric acid, boric acid, sulfurous acid, etc.) are not dissociated sufficiently to make a significant difference in the freezing point. Conse-

TABLE I.—MOLAL LOWERING OF THE FREEZING POINT (L) AND CONCENTRATION (C) ISOTONIC WITH BLOOD SERUM[a]

	L, ° C. per Mole in 1000 Gm. H_2O	C, Moles per 1000 Gm. H_2O in a Solution Isotonic with Blood Serum
Type 1A		
$L = 1.9$, $C = 0.30$		
Nonelectrolytes		
Chloral hydrate	1.87	0.298
Dextrose	1.87	0.298
Glycerin	1.87	0.298
Sucrose	1.92	0.292
Type 1B		
$L = 2.0$, $C = 0.29$		
Weak electrolytes		
Boric acid	(1.9)	...
Citric acid	1.95	0.286
Mercuric chloride	(1.8)	...
Mercuric cyanide	(1.9)	...
Tartaric acid	1.97	0.284
Type 2A		
$L = 2.0$, $C = 0.29$		
Di-divalent electrolytes		
2 = two ions, A = polyvalent anion		
Cupric sulfate	1.82	0.30
Magnesium sulfate	2.01	0.279
Zinc sulfate	2.02	0.278
Type 2B		
$L = 3.4$, $C = 0.16$		
Uni-univalent electrolytes		
2 = two ions, B = univalent anion		
Ammonium chloride	3.41	0.164
Ammonium nitrate	3.33	0.168
Potassium bromide	3.41	0.164
Potassium chloride	3.40	0.164
Potassium cyanide	3.36	0.166
Potassium iodide	3.46	0.162
Potassium nitrate	3.19	0.176
Potassium phosphate[b] (monobasic KH_2PO_4)	3.22	0.174
Silver nitrate	3.23	0.174
Sodium bicarbonate	3.56	0.156
Sodium bromide	3.49	0.160
Sodium chloride	3.44	0.162
Sodium iodide	3.59	0.156
Sodium nitrate	3.36	0.166
Type 3A		
$L = 4.3$, $C = 0.13$		
Uni-divalent electrolytes		
3 = three ions, A = polyvalent anion		
Potassium carbonate	4.51	0.124
Potassium sulfate	4.19	0.134
Sodium carbonate	4.34	0.129
Sodium phosphate (dibasic Na_2HPO_4)[b]	4.22	0.133
Sodium sulfate	4.22	0.133
Sodium thiosulfate	(4.2)	...
Type 3B		
$L = 4.8$, $C = 0.12$		
Di-univalent electrolytes		
3 = three ions, B = univalent anion		
Calcium chloride	4.82	0.116
Calcium nitrate	4.54	0.124
Cupric nitrate	(5.1)	...
Magnesium chloride	4.94	0.113
Magnesium nitrate	4.73	0.118
Zinc acetate	4.66	0.120
Zinc chloride	4.94	0.114
Zinc nitrate	4.89	0.114
Type 4A		
$L = 5.2$, $C = 0.11$		
Uni-trivalent electrolytes		
4 = four ions, A = polyvalent anion		
Potassium ferricyanide	5.28	0.106
Sodium citrate[c]	5.17	0.108
Type 4B		
$L = 6.0$, $C = 0.09$		
Tri-univalent electrolytes		
4 = four ions, B = univalent anion		
Aluminum chloride	5.69	0.098
Aluminum nitrate	6.12	0.092
Ferric chloride	6.03	0.094
Ferric nitrate	6.32	0.089
Type 5		
$L = 7.6$, $C = 0.07$		
Tetraborates		
Potassium tetraborate	7.32	0.076
Sodium tetraborate	8.0	0.070
Type M		
Miscellaneous electrolytes		
Aluminum sulfate	(4.0)	(0.14)
Chromium sulfate	4.1	(0.14)
Lead acetate	2.21	0.254
Lead nitrate	3.71	0.152
Mercuric chloride (*cf.* 1B)	(1.8)	...
Mercuric cyanide (*cf.* 1B)	(1.9)	...
Potassium aluminum sulfate	(3.5)	(0.16)
Sodium sulfide	(7.1)	(0.08)
Stannic chloride	(12.0)	(0.047)

[a] These values were read from accurate graphs similar to the one shown in Fig. 1. All data were taken from the International Critical Tables (2) unless otherwise noted. Values in parentheses are rough approximations from incomplete or inexact data.

[b] The same values for L and C apply to all hydrates because gram molecular units rather than weight units are employed. It is, of course, necessary to know the hydrate employed in making up a solution of known concentration.

[c] Hitchcock and Dougan (3).

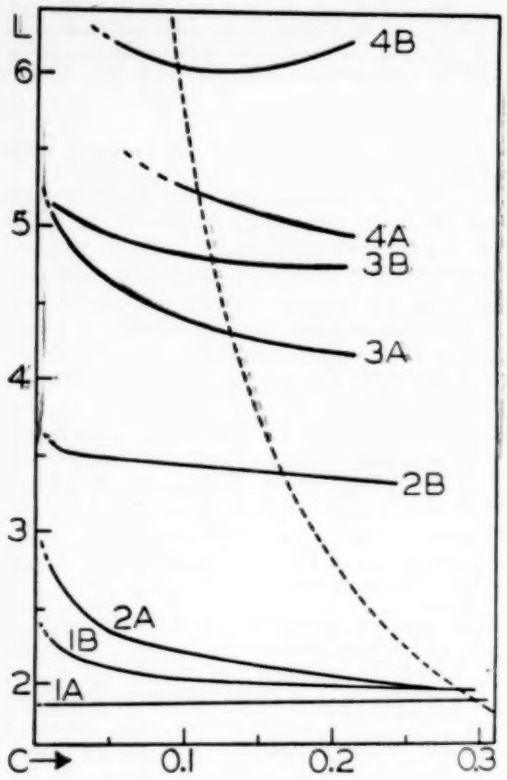


Fig. 1.—Average values of the molar lowering of the freezing point for various types of substances.

The intersection of the solid curves with the broken curve gives the average L and C values for solutions isotonic with blood serum. L = molal lowering of the freezing point. C = molal concentration.

(Zn^{++} and SO_4^{--}), the negative ion being divalent. A member of type 2B ($NaHCO_3$ or NaH_2PO_4, or NaCl) would dissociate into two ions ($Na^+ + HCO_3^-$, $Na^+ + H_2PO_4^-$, or $Na^+ + Cl^-$) but the anion would be univalent. Na_2HPO_4 would belong to type 3 because there are three ions formed as the result of the dissociation of one molecule ($2\ Na^+ + HPO_4^{--}$). Since the negative ion is divalent this compound would be assigned the letter A. Type 5 is an arbitrary classification reserved for tetraborates.

CLASSIFICATION OF COMMON DRUGS

Table II is included to show the type which would normally be assigned to a number of common drugs. These assignments were made in each case by considering the formula as it appears in standard handbooks. The rules considered in the previous section and the examples given in Table I will enable anyone to properly classify any substance. Of course, there is always the possibility that the substance may be a complex salt or weak electrolyte not shown as a member of type M in Table I. This possibility is remote, however, except in the case of colloidal preparations which may be arbitrarily placed in class 1A with a molecular weight above one thousand. If such preparations are contaminated with electrolytes it is, of course, necessary to know the amount of electrolyte present or to know the freezing point of the particular solution being used. In such cases it is probably better to calculate directly from Δt rather than to attempt classification.

THE SODIUM CHLORIDE EQUIVALENT

Many methods have been proposed for calculating the amount of salt or other substance that must be added to a hypotonic solution in order to make it isotonic with a chosen standard (1). The most convenient of these methods was suggested by Mellen and Seltzer (4) who calculate a sodium chloride coefficient for each substance, using the approximate method of Nicola (1). This coefficient is so defined that it is numerically equal to the weight

TABLE II.—CLASSIFICATION OF COMMONLY USED DRUGS ACCORDING TO IONIC TYPE

Drug	Type	Drug	Type	Drug	Type
Ammonium chloride	2B	Hyoscine hydrobromide	2B	Potassium biphosphate	2B
Antipyrine	1A	Hyoscine hydrochloride	2B	Procaine hydrochloride	2B
Atropine sulfate	3A	Lactose	1A	Silver nitrate	2B
Boric acid	1B	Magnesium chloride	3B	Sodium bicarbonate	2B
Calcium chloride	3B	Menthol	1A	Sodium biphosphate	2B
Camphor	1A	Mercuric chloride	1B	Sodium borate	5
Chlorobutanol	1A	Mercuric cyanide	1B	Sodium carbonate	3A
Cocaine hydrochloride	2B	Methenamine	1A	Sodium chloride	2B
Copper sulfate	2A	Metycaine hydrochloride	2B	Sodium iodide	2B
Dextrose	1A	Morphine hydrochloride	2B	Sodium nitrate	2B
Emetine hydrochloride	3B	Morphine sulfate	3A	Sodium phosphate	3A
Ephedrine hydrochloride	2B	Neo-synephrine hydrochloride	2B	Sucrose	1A
Ephedrine sulfate	3A	Phenacaine hydrochloride	2B	Tannic acid	1B
Epinephrine hydrochloride	2B	Phenol	1A	Tutocaine hydrochloride	2B
Ethylmorphine hydrochloride	2B	Physostigmine salicylate	2B	Urea	1B
Ethylhydrocuprein hydrochloride	2B	Physostigmine sulfate	3A	Zinc chloride	3B
Fluorescein sodium	3A	Pilocarpine hydrochloride	2B	Zinc phenolsulfonate	3B
Glycerin	1A	Pontocaine hydrochloride	2B	Zinc sulfate	2A
Homatropine hydrobromide	2B	Potassium chloride	2B		

quently acid salts of di- and tribasic acids are classified by considering the acid hydrogen as remaining attached to the negative ion. In types 2, 3 and 4 the letters A and B are used systematically, A standing for a polyvalent anion. Thus a member of type 2A ($ZnSO_4$) would dissociate into two ions of sodium chloride that would exercise the same osmotic effect as would one weight unit of the substance in question. This is a very useful quantity because the osmotic effects of different substances in solution are reduced to a common denominator, namely, the weight of sodium chloride that would

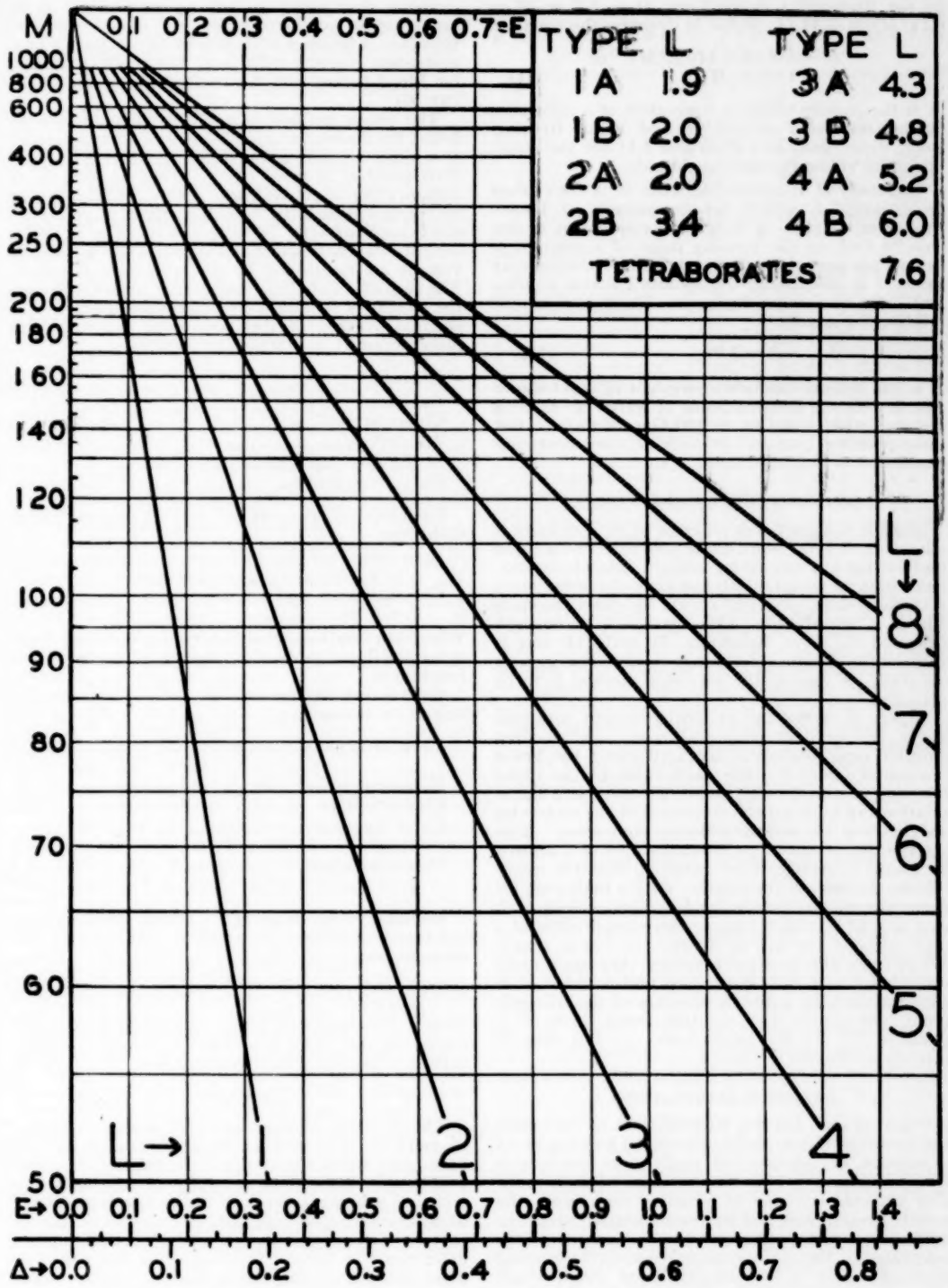


Fig. 2.—A nomograph for calculating sodium chloride equivalents.

Determine type and molecular weight from formula (see Tables I and II). Select a sloping line corresponding to the *L* value for the type. Read the *E* scale where the sloping line crosses the value of the molecular weight shown on the vertical *M* scale. To find the sodium chloride equivalent of a compound having a molecular weight of 250 and an *L* value of 3.0 locate the position of 250 on the vertical scale. Follow this line to the right until it intersects the sloping "*L* = 3" line. This point is read on the *E* scale as 0.20 which is the required value. The Δ scale gives the approximate freezing point of a 1% solution.

exert the same osmotic effect. It is a simple matter to use Equation I in the form $\Delta t = LC$ or $\Delta t = L(1/M) = 3.44\ (E/58.45)$ in deriving the equation

$$E = (58.45/3.44)(L/M), \text{ or}$$
$$E = 17.0(L/M) \qquad \text{(II)}$$

E is the sodium chloride equivalent of a substance having molecular weight M and molal freezing-point depression, L. 58.45 and 3.44 are the corresponding values for sodium chloride.

Equation II is applicable when it is convenient to estimate L values for compounds of known molecular weight. If it is more convenient in any case to look up the freezing point of a solution of known per cent concentration, this information may be used in calculating the sodium chloride equivalent. By combining Equations I and II a simple relationship results.

$$E = 1.7(\Delta t/w) \qquad \text{(III)}$$

E is the sodium chloride equivalent of a substance that lowers the freezing point of water $\Delta t°$ C. at a concentration of w Gm. in 100 Gm. of water. For most practical purposes w is the w/v per cent concentration of the substance.

RAPID CALCULATION OF E VALUES

Since it is agreed that an error of 10% or more is allowable, it is far more important to devise a rapid method for approximating sodium chloride equivalents than to spend additional time obtaining more accurate values. For this reason the chart shown in Fig. 2 is introduced. This chart is a nomograph designed to solve Equations II and III and is sufficiently accurate for most work. For greater accuracy, of course, the algebraic method is to be preferred.

Figure 2 shows the molecular weight arranged along the vertical reciprocal scale, M. Sodium chloride equivalents are arranged along the linear horizontal scale, E. For each value of the molal lowering of the freezing point, L, there is a linear relationship between the reciprocal of the molecular weights and the sodium chloride equivalent. This relationship is shown for integral values of L up to 8 by means of straight lines radiating from the upper left-hand corner of the graph. With a little practice it is easy to interpolate for fractional values of L and to read E within two- or three-hundredths of a unit without the aid of dividers or paper scales. While these aids increase accuracy they are usually not needed. If it is desired to find a value of E corresponding to a measured value of the freezing-point depression in a 1% solution, locate the freezing point in °C. on the lower scale, Δ, and read E directly above.

PRACTICAL CALCULATIONS

Figure 1 and Table I may be used to determine the concentration as well as the molal freezing-point depression of solutions of single pure substances which are isotonic with blood serum. This is probably the simplest way of handling such cases, the results being multiplied by the molecular weight in order to convert to Gm. per liter. Concentrations calculated by this method are based upon the freezing point of −0.56° C. for human blood serum which is in agreement with the standard established by Margaria (5) corresponding to a 0.95% solution of sodium chloride as compared to the U. S. P. XII value of 0.9%.

While it is customary to make collyria isotonic with tears, there seems to be no very good reason for continuing this practice inasmuch as excessive tear secretion is associated with a certain amount of irritation. On the other hand, there is good evidence that solutions isotonic with blood serum are entirely satisfactory for use in the eye (6). However, whenever it is desired to compute a concentration isotonic with tears the results obtained for blood serum may be multiplied by the factor (80/56).

The above method is not convenient for calculating the amount of a second substance to be added to a hypotonic solution in order to render it isotonic. This problem is more easily solved by the use of sodium chloride equivalents. These coefficients may be computed directly from Equations II or III or, more conveniently, by the use of Fig. 2. The weights of each substance present in a definite volume are multiplied by the respective E values. The sum of all of these products is subtracted from the weight of sodium chloride required to make the original volume of distilled water isotonic with blood serum (*i. e.*, 0.9 Gm. in 100 cc.).

Example:

Ephedrine sulfate	0.60
Chlorobutanol	0.12
Aq. dest., *q. s.*	30.00

M. Ft. Isotonic $\bar{c}$ NaCl
Sig.: Nose drops.

Calculate (E) the sodium chloride equivalent for each drug:

Ephedrine sulfate, mol. wt. 428.53, type 3a $L = 4.3$ from Fig. 2 or Equation II, $E = 0.17$.
Chlorobutanol, mol. wt. 177.17, type 1a, $L = 1.9$, $E = 0.18$.

When the sodium chloride equivalent has been determined for a drug it should be recorded for future use.

Calculation for amount of sodium chloride required for isotonicity.

30 cc. of isotonic solution contains $0.90 \times 30/100 = 0.27$ Gm. NaCl
Ephedrine sulfate $0.60 \times 0.17 = 0.10$
Chlorobutanol $0.12 \times 0.18 = 0.02$
Total equivalent represented by drugs $(0.10 + 0.02) = 0.12$ Gm. NaCl
To render solution isotonic add $(0.27 - 0.12) = 0.15$ Gm. NaCl

Perhaps some substance other than sodium chloride was desired for adjusting the tonicity, for example, dextrose.

Dextrose, mol. wt. 198.17, type 1A, $L = 1.9$, $E = 0.16$.
Divide the amount of NaCl that should have been added by the NaCl equivalent for dextrose, 0.15/0.16 = 0.94 Gm. of dextrose required.

ACCURACY

The accuracy of the results obtained by the use of methods discussed in the preceding sections is dependent upon the problem at hand. In simple problems involving solutions of pure substances for which C values are given in Table I, a high degree of accuracy can be expected. These values merely represent the molal concentration which has been determined to have a freezing point of −0.56° C. corresponding to the 0.95% sodium chloride solution determined by Margaria (5) to be isotonic with human blood serum. For the most part these values were obtained by short interpolations from cryoscopic data of high precision. When it is necessary to calculate the concentration of any of these substances which would be isotonic with the 0.9% solution of sodium chloride recommended in the U. S. P. XII no serious error will be introduced

by multiplying the molal concentration given in Table I by the factor (0.90/0.95).

When calculations are based upon the sodium chloride equivalents obtained from Fig. 2 or Equation II, the E values are independent of the standard used. Although the L values were computed for solutions isotonic with blood serum, it will be noted that they are nearly constant in the limited concentration range normally required. Thus it is possible to choose any ordinary concentration of sodium chloride as a standard, including 1.4% which is generally considered to be isotonic with tears. In the example given above the only change in the calculation would be the substitution of $(1.4 \times 30/100)$ for $(0.9 \times 30/100)$.

When exact cryoscopic data are lacking, accuracy cannot be predicted with certainty. However, a study of Table I shows that the maximum deviation for most substances is less than 10%. Naturally there is always the possibility that some compound might exhibit abnormal behavior. For the most part, however, it is safe to say that approximations made by classifying compounds according to ionic type are as dependable as those based upon routine Beckman freezing-point determinations. This point is brought out by a study of the data presented by Husa and Rossi. Table III shows all of the compounds of class 2B studied by these authors for which more exact data are available. It should not be assumed, however, that rough measurements of the freezing points of solutions are of no value. When these measurements give results that are very different from what would be predicted, a repetition of the work is indicated. In the case of physostigmine salicylate it was found that the predicted value was confirmed by a check on a different commercial sample in this laboratory. On the other hand, calcium gluconate was found to have an L value of 4.1 as compared with 3.67 calculated from the work of Husa and Rossi, or 4.8 as would be estimated from the ionic type. This indicates a tendency toward molecular association or incomplete solution in this case. It should not be assumed, however, that many salts of organic acids will behave abnormally. Estimated L values for potassium salts of acetic, oxalic, tartaric and citric acids were found to agree within 10% with values calculated from precise cryoscopic work (7).

In considering the accuracy of these estimates in the ordinary case where several different drugs are mixed together in solution and a certain amount of sodium chloride added to render the solution isotonic, several other factors must be taken into account. Uncertainty will arise as to whether L values should be computed from Fig. 1 to correspond to the exact concentration of each drug used. Because of the fact that a total error of 10% or more will not affect the quality of the final solution this is largely an academic question. However, it should be pointed out that the properties associated with activity coefficients of strong electrolytes are more dependent upon the total ionic strength of the final solution than upon the concentration of each individual substance (8). Randall and Longtin (9) show that this principle may be correctly applied to freezing points in mixtures of strong electrolytes.

In general, the accuracy required in most work can easily be obtained by using Fig. 2 and interpolating by eye. This is especially true when dealing with drugs of high molecular weight. A glance at Fig. 2 will show that when dealing with solutions of such substances at concentrations of 1% or less, all of the drug added accounts for less than 0.3 Gm. of sodium chloride in 100 cc. Since an error of 0.1 Gm. of NaCl out of 0.9 Gm. could be tolerated in most cases it is clear that a very rough visual interpolation is all that is required. Naturally when dealing with more concentrated solutions or with substances of low molecular weight the graph should be read more accurately or the algebraic method employed.

TABLE III.—A COMPARISON OF EXPERIMENTALLY DETERMINED L VALUES FOR SUBSTANCES THAT WOULD BE ASSUMED TO HAVE A VALUE OF 3.4 IN THE ABSENCE OF EXACT DATA

Compound	L Calcd. from F.-P. Data of Husa and Rossi (1)	L Calcd. from Other F.-P. Data. L. C. T. (2) Unless Noted	Per Cent Error Husa and Rossi	Per Cent Error Estn. from Type ($L = 3.4$)
Ammonium chloride	3.5	3.41	2.6	0.3
Physostigmine salicylate	4.54	3.1[a]	46.5	9.7
Potassium chloride	3.61	3.40	6.2	0.0
Potassium iodide	3.65	3.46	5.5	1.7
Potassium nitrate	3.53	3.19	10.1	6.6
Silver nitrate	3.81	3.23	17.9	5.3
Sodium bicarbonate	3.69	3.56	3.6	4.5
Sodium bromide	3.70	3.49	6.0	2.3
Sodium chloride	3.38	3.44	1.7	1.2
Sodium iodide	3.29	3.59	8.4	5.3
Sodium nitrate	3.06	3.36	8.9	1.2

[a] Determined experimentally in this laboratory by a modification of the method of Hitchcock and Dougan (3). As a check on the method, the freezing point of a 0.05 M solution of KCl was found to agree within 3% with values given in the International Critical Tables (2) for this concentration of KCl.

SUMMARY

It is shown that substances of the same ionic type have nearly the same molal freezing-point depression. This fact is used as the basis of estimating approximate isotonic concentrations of substances for which freezing-point data are lacking. Equations are derived that are applicable in all cases whether approximations or exact cryoscopic data are employed. These equations can be used to compute the weight of sodium chloride that would have the same osmotic effect as would 1 Gm. of the substance under consideration. A graphic method is provided for rapidly calculating this sodium chloride coefficient, either from ionic type and molecular weight, or from the freezing point of 1% solutions. The accuracy of the method is discussed and practical calculations of amounts of different substances required to prepare isotonic solutions are described.

REFERENCES

(1) Husa, W. J., and Rossi, O. A., THIS JOURNAL, 31 (1942), 270.

(2) "International Critical Tables," Vol. IV, McGraw-Hill Book Co., New York, 1928, p. 254.

(3) Hitchcock, David I., and Dougan, Ruth B., *J. Gen. Physiol.*, 18 (1935), 485.

(4) Mellen, M., and Seltzer, L. A., THIS JOURNAL, 25 (1936), 759.
(5) Margaria, R., *J. Physiol.*, 70 (1930), 417.
(6) Hind, H. W., (private communication).
(7) Roth, W. A., and Schiel, K., "Landolt-Börnstein Physikalisch Chemische Tabellen," Julius Springer, Berlin, Eg. II, 1931, p. 1473.
(8) Lewis, G. N., and Randall, M., "Thermodynamics," McGraw-Hill Book Company, New York, 1923, p. 380.
(9) Randall, M., and Longtin, B., *J. Phys. Chem.*, 44 (1940), 427.

Age of Animals and Drug Action*,†

By K. K. Chen and E. Brown Robbins‡

In the present series of experiments, 8 drugs were studied in guinea pigs, rats and mice, with the aim of ascertaining the influence of animal age upon the action of drugs. The substances employed were histamine dihydrochloride, aconitine hydrobromide, morphine sulfate, sodium sulfapyridine, "Seconal Sodium" (Sodium Propyl-methyl-carbinyl Allyl Barbiturate, Lilly), ephedrine hydrochloride, picrotoxin and senecionine. The last compound is an alkaloid of *Senecio integerrimus*, which causes death by producing liver necrosis (1, 2). Injections were made both subcutaneously and intravenously into guinea pigs with histamine dihydrochloride; subcutaneously into guinea pigs with aconitine hydrobromide; intravenously into rats with morphine sulfate, sodium sulfapyridine and ephedrine hydrochloride; intraperitoneally into rats with "Seconal Sodium"; and intravenously into mice with picrotoxin and senecionine. Appropriate solutions were prepared in order to avoid an excessive volume of each dose. The age groups of guinea pigs, all males, varied from 10 days to 1 yr. The age of rats varied from 2 wks. to 2 yrs. Female rats were injected with morphine sulfate and ephedrine hydrochloride, but both sexes were employed for sodium sulfapyridine and "Seconal Sodium." The age groups of mice varied from $1^1/_2$ mos. to 2 yrs., both sexes being used.

As usual, different dose increments of each drug were administered to various age groups of the selected animal with the purpose of determining the median doses—be they lethal, anesthetic or convulsive. As shown in Table I, the total number of animals injected varied from 15 to 129 for the estimation of each dose. The median doses as listed in the last column were all computed by the combined slope according to the method of Miller, Bliss and Braun (3), except that with ephedrine hydrochloride the χ^2 value exceeded the 5% point. The median lethal doses of ephedrine hydrochloride were therefore calculated by their individual slopes.

By the comparison of median lethal doses (LD_{50}'s), it becomes obvious that no difference of response occurs in guinea pigs of various ages when histamine dihydrochloride or aconitine hydrobromide is injected subcutaneously. However, if histamine dihydrochloride is given by vein (saphenous), the 10-day-old pigs are significantly less susceptible than older ones. An opposite effect is manifested by morphine sulfate when administered intravenously into female rats. The 14-day-old animals are decidedly more susceptible than older ones. This would support the contention of clinicians that morphine is more toxic to children than to adults. With sodium sulfapyridine, the 1- and $1^1/_2$-mo.-old rats are less susceptible than older ones. The median anesthetic dose (AD_{50}) of "Seconal Sodium" is smallest in the 2-yr.-old group, and largest in the $1^1/_2$-mo. group, showing that senile rats are the most sensitive and very young animals the least sensitive. The results with ephedrine hydrochloride are very irregular. Rats aged 1 and $1^1/_2$ mos. are definitely more susceptible than those immediately younger or older, namely, those aged 2 wks. or 2 mos. The apparently small figures for the 6- and 12-mo. age groups cannot be considered as evidence of high susceptibility, because the differences between them and the adjacent doses are not statistically significant, their standard errors being unusually large. Regarding picrotoxin, examination of the median convulsive doses (CD_{50}'s) reveals that mice aged 2 yrs. are more susceptible than younger groups of mice. The LD_{50} of

* Received July 28, 1943, from the Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Ind.
† The authors are indebted to Mr. John C. Hanson for his valuable assistance in the statistical analysis of our data.
‡ Now Lieutenant, U. S. N. R.

senecionine may be taken as an index of liver injury in the various age groups of mice studied. There is no significant difference between successive figures. In other words, age does not exert any influence on the animal's response to this alkaloid. older ones; and sodium sulfapyridine, less toxic to 1- and $1^1/_2$-mo.-old rats than to older ones. Similarly, "Seconal Sodium," administered intraperitoneally, seems to be less effective in producing anesthesia in the $1^1/_2$-mo.-old rats than in older rats. In

TABLE I.—ANIMAL AGE AND DRUG ACTION

Drug	Route of Administration	Reaction Studied	Animal Used	Sex	Age of Animals Mos.	Age of Animals Days	Number Injected	Median Dose ± Standard Error, Mg./Kg.
Histamine dihydrochloride	Subcutaneous	LD_{50}	Guinea pigs	M		10	25	3.445 ± 0.315
						18	24	3.753 ± 0.348
					1		32	3.199 ± 0.242
					2		34	2.815 ± 0.219
					3		29	3.496 ± 0.295
					6		40	4.706 ± 0.383
					12		30	3.803 ± 0.435
Histamine dihydrochloride	Intravenous	LD_{50}	Guinea pigs	M		10	76	0.4395 ± 0.0324
					2		44	0.3293 ± 0.0214
					7		48	0.3204 ± 0.0219
					12		30	0.2943 ± 0.0247
Aconitine hydrobromide	Subcutaneous	LD_{50}	Guinea pigs	M		10	30	0.06073 ± 0.00345
						18	30	0.05868 ± 0.00338
					1		28	0.05888 ± 0.00362
					2		30	0.06117 ± 0.00347
					3		30	0.06096 ± 0.00356
					6		30	0.06043 ± 0.00376
Morphine sulfate	Intravenous	LD_{50}	Rats	F		14	100	119.1 ± 8.5
					1		129	280.0 ± 19.0
					$1^1/_2$		50	282.5 ± 26.4
					2		97	285.0 ± 22.0
					4		35	253.2 ± 33.1
					6		52	220.1 ± 24.6
					12		30	171.6 ± 20.3
					24		27	195.5 ± 26.2
Sodium sulfapyridine	Intravenous	LD_{50}	Rats	M and F	1		15	930.7 ± 32.8
					$1^1/_2$		30	881.2 ± 23.5
					6		18	648.0 ± 25.7
					12		18	719.3 ± 22.8
					24		21	716.0 ± 23.0
"Seconal Sodium"	Intraperitoneal	AD_{50}	Rats	M and F	$1^1/_2$		30	50.74 ± 2.44
					6		42	41.66 ± 1.99
					12		51	42.04 ± 1.61
					24		40	34.34 ± 1.49
Ephedrine hydrochloride	Intravenous	LD_{50}	Rats	F		14	53	180.5 ± 4.87
					1		61	82.85 ± 6.54
					$1^1/_2$		48	82.58 ± 7.65
					2		51	165.5 ± 14.7
					4		52	112.2 ± 22.4
					6		49	69.88 ± 5.07
					12		30	69.23 ± 30.5
					24		24	112.1 ± 5.8
Picrotoxin	Intravenous	CD_{50}	Mice	M and F	$1^1/_2$		40	1.404 ± 0.073
					6		40	1.302 ± 0.065
					12		40	1.385 ± 0.072
					24		30	1.064 ± 0.064
Senecionine	Intravenous	LD_{50}	Mice	M and F	$1^1/_2$		40	68.13 ± 3.98
					6		40	68.85 ± 4.17
					12		41	79.87 ± 9.58
					24		34	71.63 ± 6.74

From the above data, it is clear that no generalization can be made concerning the influence of age upon the action of drugs. Each compound appears to have its own characteristics. When injected intravenously, histamine dihydrochloride is less toxic to 10-day-old guinea pigs than to contrast, morphine sulfate when injected by vein into rats is most toxic to the youngest age group, namely, 14-day-olds. Other drugs are relatively more active in senile animals. Thus, it takes a smaller dose of "Seconal Sodium" to anesthetize rats 2 yrs. of age than the younger animals. Also,

it requires less picrotoxin to cause convulsions in mice 2 yrs. of age than in younger ones.

SUMMARY

1. Of 8 drugs studied in the various age groups of animals, histamine dihydrochloride when injected intravenously is less toxic to guinea pigs 10 days old than to older ones. No significant differences in LD_{50}'s attributable to age influence occur when histamine dihydrochloride or aconitine hydrobromide is injected subcutaneously into guinea pigs.

2. Morphine sulfate given by vein is more toxic to rats 14 days old than to older ones. Sodium sulfapyridine, on the other hand, is better tolerated by rats 1 to $1^1/_2$ mos. old than by those 6 mos. of age.

3. Rats 2 yrs. old are the most, and those $1^1/_2$ mos. old, the least susceptible to intraperitoneal injection of "Seconal Sodium" in production of anesthesia. Rats aged 1 to $1^1/_2$ mos. succumb to a smaller dose of ephedrine hydrochloride, injected intraveneously, than those younger or older, namely, 14 days or 2 mos. old.

4. Mice 2 yrs. of age are more sensitive to the convulsive action of picrotoxin than younger mice. No age effect can be demonstrated in mice by intravenous injection of senecionine, which causes death by producing liver necrosis.

5. Results of our experiments indicate that it is impossible to predict how far the age of animals will influence the drug action, each substance having its own characteristics.

REFERENCES

(1) Manske, R. H. F., *Can. J. Res.*, B17 (1939), 1.
(2) Harris, P. N., Anderson, R. C., and Chen, K. K., *J. Pharmacol.*, 75 (1942), 69.
(3) Miller, L. C., Bliss, C. I., and Braun, H. A., THIS JOURNAL, 28 (1939), 644.

A General Theory for the Mode of Action of the Sulfonamides*

By S. W. Lee and E. J. Foley

The assumption has frequently been made that the sulfonamides exert their bacteriostatic effect through reaction with one or more enzyme systems of the organisms. A great deal of factual information points to the generality of this effect of the drugs. The sulfonamides have been found to interact with several enzymes, some of them in an isolated state, usually lowering their activity. Sulfanilamide, together with several of its functional and related derivatives, has been shown to diminish the catalytic activity of catalase, both *in vitro* (1, 2), and in a series of treated patients (3). In addition, Carrara (2) found the activity of peroxidase to be inhibited by sulfanilamide. Zeller (4) has studied the similar effect upon cholinesterase, and has commented upon the reversibility of the interaction. In an extensive study, Johnson has shown the inhibitory effects of sulfanilamide and other drugs on luciferase. His work included experiments on isolated luciferase, as well as on luminous bacteria, in which the luciferin-luciferase system was present. By studying this system as a function of temperature, Johnson, *et al.* (5), have found facts which are best explained by treating the reaction of the sulfonamide on the enzymes as a reversible denaturation or, more specifically, as a denaturation of one of two (native and denatured) forms of the enzymes normally present. The existence of the two forms of the enzyme and the temperature reversibility of the enzymatic activity have been pointed out by Northrup, *et al.*, in work on pepsin and other enzymes.

The actual identity of the bacterial enzyme, or enzymes, affected by the sulfonamides in such a manner as to diminish growth, is unsettled. Fildes (6) has stated that the role of the sulfonamides is that of competitors of essential metabolites for an enzyme. Others have felt that the anticatalase action accounts for their effects, but this stand is found untenable by Sevag (7), who has suggested that these drugs interfere with, or block, a bacterial enzyme system specifically concerned with respiration of the cell.

Now, if these results be taken to indicate the existence of interactions between bacterial enzyme systems and the sulfonamides (and other drugs) and, further, that these interactions alter the functioning abilities of the enzymes, a clear and consistent

* Received July 23, 1943, from Wallace Laboratories, Inc., New Brunswick, N. J.

picture may be drawn of the manner in which the sulfonamides exert their influence. Any acceptable theory for the mode of action of these drugs must explain their activity alone, and their conjoint actions with other drugs.

The action of the sulfonamides alone may be somewhat as follows: Consider the enzyme in question to consist of a mosiac of reactive, or receptive groups. Some of these reactive groups might have an affinity for the aminophenyl radical and others, one for the amido linkage, and so on. The nature of the force which brings about this binding of the various foreign molecules to the enzyme is ill defined, but it must be a weak, adsorptive one. The weakness is attested to by the easy reversibility (4, 5) of most of the actions of the neutral sulfonamides. Whatever the nature of this binding force, the effect on the enzyme is most likely comparable to a partial local denaturation. The reaction must be partial and local because of its reversibility, and its temperature coefficient (see Table I and reference 5) indicates it to be a denaturation. Thus, by this kind of action on the enzyme, the sulfonamides decrease its catalytic activity. It might be pointed out that this local action on the enzyme need not always lead to an impairment of activity. Lamanna (8) claims that the growth of many organisms is stimulated by low concentrations of sulfanilamide.

The coaction of sulfonamides with other drugs may be divided into antagonism and potentiation. The substances which antagonize the sulfonamides are various simple organic compounds and complex proteins. The coaction of many simple substances with the sulfonamides has been studied by Kohn and Harris (9) and others. The best known antagonistic compound is *p*-aminobenzoic acid, which has been exhaustively studied (10). McIlwain (11) has formed the hypothesis that "the antibacterial action of sulfanilamide is due to its competitive inhibition of enzymes which normally interact with *p*-aminobenzoic acid or a closely related substance." *p*-Aminobenzoic acid may be used to typify the simple substances capable of interfering with the action of the sulfonamides, and to us, the "competitive inhibition" effect comes about as follows: This compound may be considered to have a greater affinity for the "sulfonamide-receptive" groups than do the sulfonamides. This greater total affinity might be due to higher ionization of the *p*-aminobenzoic acid (12) or to stronger adsorptive forces. If the *p*-amino benzoic acid be more strongly, and hence preferentially, adsorbed, one would expect it to protect the organism from a large molecular excess of sulfanilamide, and such is the case. It is shown in Table I that *p*-aminobenzoic acid, in the concentration used, slightly accelerates the growth of *E. coli*, at 37° and lower, indicating a stimulatory combination of the acid with the enzymes of the organism. Of much more importance to the point in question is the marked toxic effect of this substance at slightly higher temperatures. In other words, the irreversible change of the enzyme is greatly accelerated by the low (10^{-5} *M*) concentration of *p*-aminobenzoic acid. This is further proof of the existence of the enzyme–*p*-aminobenzoic acid combination. It is also of interest to note that the inhibitory action of *p*-aminobenzoic acid alone does not increase as much with temperature as does that of sulfathiazole. This is in line with its lower denaturizing tendencies, which are entirely absent at low temperatures. This same mechanism can, of course, be extended to cover the other sulfonamide inhibitors which are simple organic compounds and to explain the mutual specificity between the various sulfonamides and these structurally related "substrates" (11).

A corollary of this concept explains the inhibitory action of various protein substances, such as serum, crystalline egg albumin, humin (8) and the many substances in various inhibitory media (13). These substances afford additional "sulfonamide-receptive" groups, and thus effectively dilute the sulfonamide.

Many substances have been described (9, 14) which, in concentrations at which they exert no ill effects on the growth of the organism, increase the bacteriostatic activity of the sulfonamides. Little has been said about the nature of this effect. One logical explanation might be that they alter the nature of the cell wall, and its permeability. We have carried out preliminary experiments on *E. coli* with several anionic and nonionic wetting agents, and find none of them to potentiate sulfathiazole. It would appear again that the reaction is upon the enzymes of the organism. Consider that the potentiating substance (such as urea or adenine; see Table I) is attached to other receptive groups than those occupied by the sulfonamides. This combination (or local denaturation), unlike that with the sulfonamides, does not diminish the activity of the enzyme at the optimum or lower temperatures (see Table I). Urea and adenine increase the growth rate in the mentioned temperature range.

At temperatures higher than 37°, urea and adenine greatly diminish rates by increasing the irreversible denaturation. If sulfathiazole be added to the above systems, it becomes attached to the enzymes in its usual positions, and exerts its local effects. Now, if this denaturation be in addition to that of the potentiators, the total diminution of the activity of the enzyme is greater than additive, and the resulting effect is one of synergism, or of potentiation of the sulfonamide. In other words, the two separate denaturations render the enzyme much less capable of carrying out its catalytic functions. At temperatures higher than 37° this effect becomes much more irreversible and complete.

TABLE I.—THE EFFECT OF VARIOUS DRUGS AND SOME OF THEIR COMBINATIONS ON THE GROWTH RATE OF *E. coli* AS A FUNCTION OF TEMPERATURE

Medium	Temperature 20°	30°	34°	37°	39°	42°	44°
Control (SG)	0.05	0.08	0.15	0.16	0.16	0.12	0.08
Sulfathiazole (STA)	0.04	0.06	0.06	0.065	0.07	0.05	0.01
p-Aminobenzoic acid (PAB)	0.06	0.09	0.16	0.15	0.16	0.10	0.05
Adenine	0.06	0.09	0.15	0.14	0.125	0.07	..
Urea 1%	0.05	0.08	0.16	0.17	0.12	0.05	0.0
STA-PAB	0.06	0.09	0.13	0.14	0.16	0.10	0.04
STA-Adenine	..	0.045	0.045	0.045	0.04	0.02	..
STA-Urea	..	0.04	0.045	0.048	0.05	0.03	0.0
STA-Adenine-Urea	..	0.038	0.04	0.042	0.035	0.02	..
STA-PAB-Urea	0.04	0.08	0.16	0.14	0.14	0.04	0.02
Control (SG)	0.05	0.085	0.15	0.15	0.16	0.12	0.08

All concentrations, except that of urea, are 10^{-3} M. An inoculum was used which gave an initial population of about 50 million organisms per ml. The readings were taken after 5 hr. of growth in a salt-glucose (SG) medium (9). The figures given are density readings from the drum of a Coleman universalt spectrophotometer. The turbidity readings of this organism very nearly obey Beer's Law.

If this be the manner in which the sulfonamides act upon the enzyme systems, one would expect that other drugs will be found which are either more strongly adsorbed than the sulfonamides or, because of their structural dissimilarity, become adsorbed at other positions. In either case, these drugs would not be inhibited by *p*-aminobenzoic acid. To this group, perhaps, belong the diamidines (15). They are reported to be a promising group of compounds which is not antagonized by *p*-aminobenzoic acid.

In summary, a general theory for the mode of action of the sulfonamides has been put forward. It is based on an extension of the known interaction of these drugs with bacterial and other enzymes. The sulfonamide is considered to cause local damage (denaturation) to certain sulfonamide-receptive parts of the enzyme mosaic. The conjoint action of these drugs with others is explained similarly. The other drugs, potentiators or inhibitors as the case may be, become adsorbed at the same or other receptive positions and either protect the enzyme from the sulfonamide (if they be at the sulfonamide positions) or enhance its activity (if they be at other positions). Both a review of the work of others and reported temperature studies of the above separate and combined effects give considerable weight to these views.

REFERENCES

(1) Collier, H. B., *Can. J. Research*, 18 (1940), 345.
(2) Carrara, G., and Chiancone, F. M., *Chimica e industria (Italy)*, 23 (1941), 435.
(3) Clyman, M., *Ann. Int. Med.*, 14 (1940), 406.
(4) Zeller, E. A., *Helv. Chim. Acta*, 25 (1942), 216.
(5) Johnson, F. H., Eyring, H., and Williams, R. W., *J. Cellular Comp. Physiol.*, 20 (1942), 247.
(6) Fildes, P., *Lancet*, 1 (1940), 955.
(7) Sevag, M. G., and Shelburne, M., *J. Bact.*, 43 (1942), 411 (and subsequent papers).
(8) Lamanna, C., *Science*, 95 (1942), 304.
(9) Kohn, H. I., and Harris, J. S., *J. Pharmacol.*, 77 (1943), 1.
(10) Woods, D. D., *Brit. J. Exptl. Path.*, 21 (1940), 74.
(11) McIlwain, H., *Science*, 95 (1942), 509.
(12) Cowles, P. B., *Yale J. Biol. Med.*, 14 (1942), 599.
(13) McLeod, C. M., and Mirick, G. S., *J. Bact.*, 44 (1942), 277.
(14) Tsuchiya, H. M., Tenenberg, D. J., Clark, W. G., and Strakosch, E. A., *Proc. Soc. Exptl. Biol. Med.*, 50 (1942), 262.
(15) *J. Am. Med. Assoc.*, 121 (1943), 946.

Spectrophotometric Studies on the Sulfonamides*

By Joseph L. Ciminera and Paul W. Wilcox

Ultraviolet absorption spectra of several sulfonamides in various solvents have been reported by other investigators (1, 2, 3, 4). In this paper are presented the ultraviolet absorption spectra in distilled water of seventeen sulfonamides, including their acetyl derivatives. A procedure has been devised for the qualitative differentiation of these sulfonamides, as well as a method for their quantitative estimation. Sulfonamides may be determined colorimetrically by several well-known methods, but a spectrophotometric procedure should prove more accurate as well as less time consuming since it eliminates the need for the development of a colored compound. Although the data accumulated apply only to the determination of pure sulfonamides in distilled water, the possibility of extending the determination to other solvents and to various body fluids will receive attention. One application of the method described herein was to check the solubility of the sulfonamides in distilled water at 20° and 37.5° C., a measurement which had been made previously on the same solutions, both gravimetrically and colorimetrically.

EXPERIMENTAL

Commercial samples of the sulfonamides were purified by recrystallization from appropriate solvents and dried to constant weight at 100° C. Solutions containing 12.50 mg./L. in distilled water were used for determining the absorption curves of all the compounds described herein excepting 2-sulfanilamido-4-methylpyrimidine, where a concentration of 12.55 mg./L. was employed. Solution was effected by constant agitation and gentle warming in a water bath. The dilutions employed for determining concentration curves at a predetermined wave length are given in Table I. Distilled water was used as a blank.

Spectrophotometric determinations were made using the Beckman Quartz Spectrophotometer, Model DU. Matched, fused quartz absorption cells, 1 cm., square type, supplied by the National Technical Laboratories were used for this study. The source of ultraviolet light was a hydrogen discharge tube supplied by the same laboratories. The spectral range studied was λ220–λ320 mμ, except in the case of sulfapyridine and its acetylated derivative, where the range studied was λ220–λ345 mμ. In a few cases, measurements as low as λ217 mμ were made. Readings were taken 5 mμ apart except at the indicated minima and maxima where the increment used was 1 mμ. The effective slit width was varied from 0.25 mm. (nominal band width 1.2 mμ) at 320 mμ to 2.0 mm. (nominal band width 2.8 mμ) at 220 mμ. These slit adjustments were made to give the minimum band width consistent with a full-scale reading of the galvanometer indicating meter. Results obtained with this type instrument at the National Technical Laboratories demonstrated that the wave-length scale was accurate and reproducible to better than 1 mμ (10). A special RCA C7032 phototube is used for measurements in the ultraviolet region of the spectrum.

The ultraviolet absorption curves are shown in Figs. 1 to 6. Figure 7 shows a typical concentration curve. Table I presents pertinent data necessary for the qualitative and quantitative determination of the seventeen sulfonamides.

Definition of Terms (5):

C. I. = Concentration range investigated.
C. B. = Concentration range following the Beer-Lambert law within ±2%.
D = Density or extinction (as read from the density scale of the instrument),
= $\log I_0/I$,
where I_0 = incident intensity, I = transmitted intensity.
k = Extinction coefficient,
= $1/dc \cdot \log I_0/I$,
where d = cell thickness in cm., c = concentration in Gm./L.
ϵ = Molecular extinction coefficient,
= $k \times$ mol. wt.
P. E. = Probable error (6),

$$= 0.67449 \sqrt{\frac{S(x - \bar{x})^2}{n(n-1)}}$$

where $S(x - \bar{x})^2$ = sum of the squares of deviations from the mean, n = number of values used in the calculation of the mean.
λ = Wave length in mμ.
$\bar{k}_{\lambda\ max.}$ = Average maximum extinction coefficient.
$\log \bar{\epsilon}_{\lambda_{max.}}$ = Average maximum log molecular extinction coefficient.

The Beer-Lambert law is expressed by the equation (5)

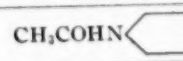
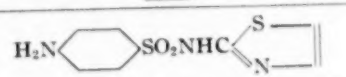
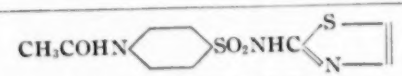
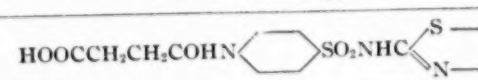
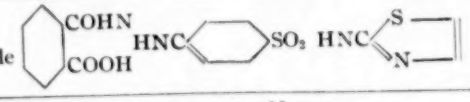
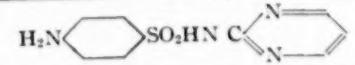
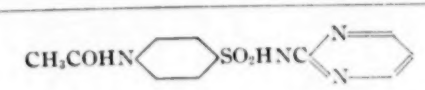
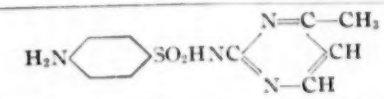
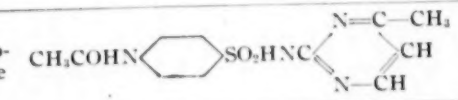
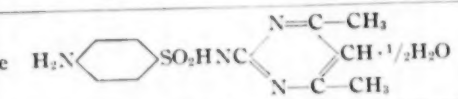
$$I = I_0 \cdot 10^{-kcd}$$

where k is a constant for the wave length and compound under consideration.

To determine over what range of concentration the Beer-Lambert law may be applied, the extinction coefficient (k) was determined for each of the concentrations investigated at the point of maximum absorption (λ max.). An average value for k was then calculated; none of the individual k values employed in its calculation varied more than ±2% from the average. In order to do this, some values had to be eliminated; the values eliminated were usually those obtained with the lower concentrations studied. The 2% variation allowed is purely an arbitrary quantity which was found to give an almost linear relationship between density or extinction and the log

* Received July 3, 1943, from the Pharmaceutical Research Laboratories, Medical Research Division, Sharp & Dohme, Inc., Glenolden, Pa.

TABLE I.—SPECTROSCOPIC PROPERTIES OF SEVENTEEN SULFONAMIDES IN DISTILLED WATER

Compound	Formula	M. W.	λ max. (mμ)	λ min. (mμ)	C. I. (Mg./L.)	C. B. (Mg./L.)	$\bar{k}_{\lambda}$ max. ±3.5 × P. E.	log $\bar{\epsilon}_{\lambda}$ max. ±3.5 × P. E.	Absorption Ratio, $D_{\lambda_1}/D_{\lambda_2}$
Sulfanilamide		172.14	258	224	2.5–15	2.5–15	92.9 ± 0.5 (258 mμ)	4.204 ± 0.002 (258 mμ)	2.00 (258/275 mμ)
Acetylsulfanilamide		214.24	255–257	...	2.5–15	5.0–15	90.3 ± 1.0 (256 mμ)	4.287 ± 0.004 (256 mμ)	2.40 (256/275 mμ)
Sulfathiazole		255.306	258 283–284	227 266–267	2.5–15	5.0–15	74.5 ± 1.0 (283.5 mμ)	4.279 ± 0.006 (283.5 mμ)	1.18 (283.5/258 mμ)
Acetylsulfathiazole		297.306	258 281	224 267	0.5–15	5.0–15	65.9 ± 0.8 (258 mμ)	4.292 ± 0.005 (258 mμ)	0.95 (281/258 mμ)
'Sulfasuxidine' Succinylsulfathiazole		355.4	258–259 281–283	224–226 267	0.5–15	2.5–15	55.5 ± 0.5 (282 mμ)	4.295 ± 0.004 (282 mμ)	1.00 (282/258.5 mμ)
'Sulfathalidine' Phthalylsulfathiazole		403.418	257 281–285	228–231 265	2.5–15	5.0–15	51.0 ± 0.3 (283 mμ)	4.313 ± 0.003 (283 mμ)	1.21 (283/257 mμ)
Sulfadiazine		250.3	239–243 263–264	230 244	0.5–15	2.5–15	72.2 ± 0.4 (263.5 mμ)	4.257 ± 0.033 (263.5 mμ)	1.53 (263.5/241 mμ)
Acetylsulfadiazine		292.3	243 260–261 302–307	246 300–301	0.5–15	5.0–15	76.0 ± 0.4 (260.5 mμ)	4.346 ± 0.002 (260.5 mμ)	1.23 (260.5/243 mμ) 9.10 (260.5/304.5 mμ)
2-Sulfanilamido-4-methylpyrimidine (Sulfamerazine)		264.3	239–241 263–264	224 246–248	1.0–15	2.5–15	70.8 ± 1.0 (263.5 mμ)	4.272 ± 0.006 (263.5 mμ)	1.25 (263.5/240 mμ)
2-Acetylsulfanilamido-4-methylpyrimidine		306.3	240–243 260–263 304–308	220 247–248 295	1.0–15	7.5–15	74.4 ± 0.5 (262 mμ)	4.358 ± 0.003 (262 mμ)	1.14 (262/241 mμ) 5.04 (262/306 mμ)
2-Sulfanilamido-4,6-dimethylpyrimidine (Sulfamethazine)		287.31	241 260–262	222 248–249	2.5–15	2.5–15	66.4 ± 0.3 (262 mμ)	4.280 ± 0.002 (262 mμ)	1.06 (262/241 mμ)
		320.31	240–242 262	248–249 295	2.5–15	7.5–15	70.3 ± 0.2 (262 mμ)	4.353 ± 0.001 (262 mμ)	1.05 (262/241 mμ)

2-Acetylsulfanilamido-4,6-dimethylpyrimidine	CH_3COHN–C_6H_4–SO_2HNC(=N–C(=CH–C(=N–)–CH_3)–CH_3)	320.31	240–242 262 302–303	248–249 295	2.5–15	7.5–15	70.3 ± 0.2 (262 mμ)	4.353 ± 0.001 (262 mμ)	1.05 (262/241 mμ) 3.32 (262/302.5 mμ)
Sulfaguanidine	H_2N–C_6H_4–SO_2N=C(NH_2)–NH_2 · H_2O	232.0	259	227	2.5–15	2.5–15	81.6 ± 1.0 (259 mμ)	4.277 ± 0.005 (259 mμ)	1.90 (259/275 mμ)
Acetylsulfaguanidine	CH_3COHN–C_6H_4–SO_2N=C(NH_2)–NH_2 · H_2O	274.0	258	226	1.0–15	1.0–15	80.1 ± 0.4 (258 mμ)	4.341 ± 0.002 (258 mμ)	2.07 (258/275 mμ)
Sulfapyridine	H_2N–C_6H_4–SO_2HN–HNC_5H_3N	249.17	243–245 260–262 311–313	223 250 295	1.0–15	2.5–12.5	69.9 ± 0.4 (261 mμ)	4.241 ± 0.002 (261 mμ)	1.86 (261/312 mμ) 1.12 (312/295 mμ)
Acetylsulfapyridine	CH_3COHN–C_6H_4–SO_2HN–HNC_5H_3N	291.17	243 260 311–312	249 290	0.5–15	1.0–15	66.3 ± 0.7 (260 mμ)	4.285 ± 0.004 (260 mμ)	1.83 (260/311.5 mμ) 1.59 (311.5/290 mμ)
p-Caproylaminobenzenesulfonhydroxamide	$C_5H_{11}COHN$–C_6H_4–SO_2NHOH	286.34	254–257	222	2.5–15	2.5–15	54.8 ± 0.4 (255.5 mμ)	4.196 ± 0.004 (255.5 mμ)	2.20 (255.5/275 mμ)

concentration of the solution. A numerical example follows:

2 - ACETYLSULFANILAMIDO - 4, 6 - DIMETHYLPYRIMIDINE (IN DISTILLED WATER AT 2620 Å.)

Concn. Mg./L.	Log Concn.	Extinction, D	k	$k-\bar{k}^a$	$\%(k-\bar{k})$
15.0	1.1761	1.055	70.3	0.0	0.0
12.5	1.0969	0.883	70.6	0.3	0.4
10.0	1.0000	0.701	70.1	−0.2	−0.3
7.5	0.8751	0.527	70.3	0.0	0.0
5.0	0.6990	0.363	72.6	...	...
2.5	0.3979	0.184	73.6	...	...

[a] $\bar{k}$ = 70.3.

Thus, it can readily be seen that 2-acetylsulfanilamido-4,6-dimethylpyrimidine follows the Beer-Lambert law for concentrations of 7.5 to 15.0 mg./L. within the limits ±2%.

We have, therefore, two methods for determining the concentration of an unknown aqueous solution of a pure sulfonamide. We may either calculate the concentration from the average extinction coefficient (for c (Gm./L.) = D/k for a 1-cm. cell) utilizing the probable error or read off the concentration directly from the log concentration curve.

For each value of $\bar{k}_{\lambda\ max.}$ and log $\bar{\epsilon}_{\lambda\ max.}$ the probable error was calculated and multiplied by ±3.5 to assure ourselves odds of 50 to 1 against the occurrence of deviations greater or less than the average values calculated (7).

It may be seen from Fig. 1 that all the sulfonamides studied have a maximum in the region λ254–λ264 mμ. From Table I it will be seen that the sulfanilamides and the sulfaguanidines have but one maximum in the region λ255–λ259 mμ. The sulfathiazoles have a maximum in the region λ257–λ259 mμ and one in the region λ281–λ285 mμ. The 2-sulfanilamidopyrimidines have a maximum in the regions λ239–λ243 mμ and λ260–λ264 mμ, and their acetylated forms show, in addition, a maximum in the region λ302–λ308 mμ. The sulfapyridines show three maxima in the regions λ243–λ245, λ260–λ262 and λ311–λ313 mμ. Although these maxima may be of some value in differentiating the several classes of compounds, they do not differ sufficiently in most individual cases to be of value in definitely identifying one or more closely related compounds.

A criterion of identity that appears to be more dependable than the wave length of the maxima is the absorption ratio (8, 9). Although this is generally defined as the ratio of the extinction at two arbitrarily selected wave lengths on either side of an absorption maximum, the selection of any two

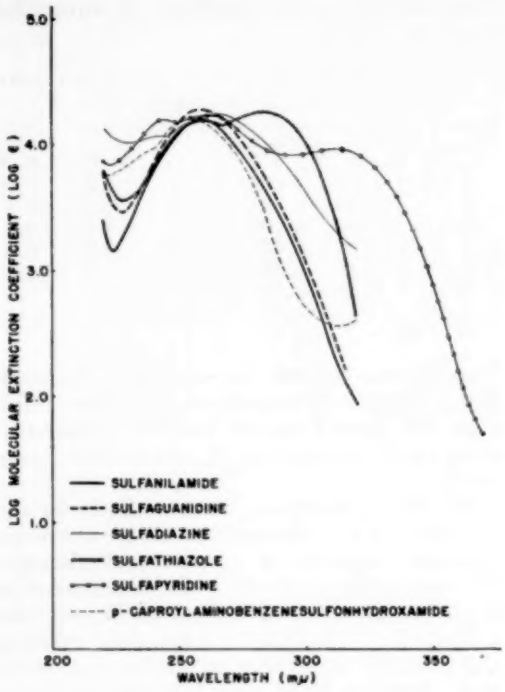


Fig. 1.—Absorption spectra of several classes of sulfonamides.

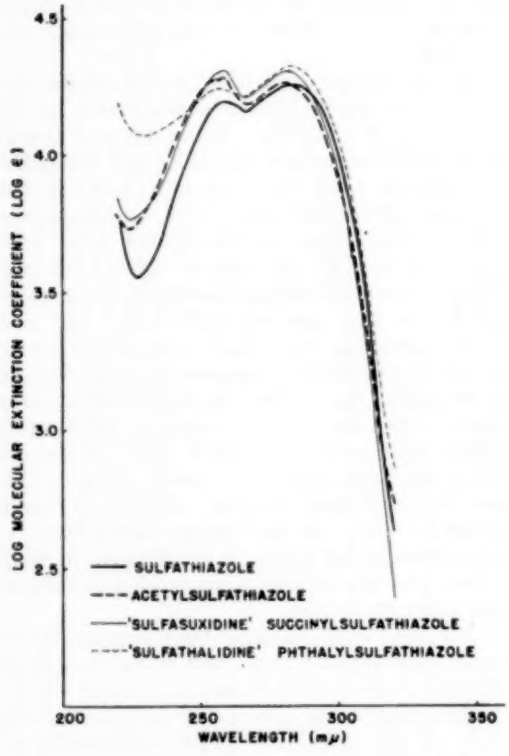


Fig. 2.—Absorption spectra of the sulfathiazoles in distilled water.

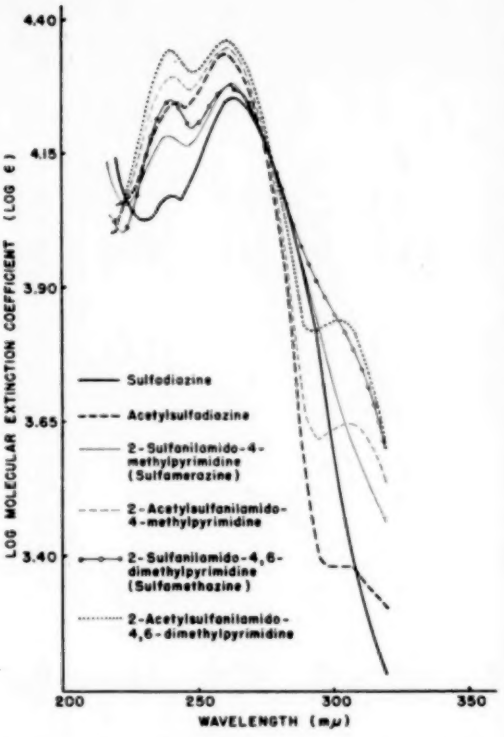


Fig. 3.—Absorption spectra of the 2-sulfanilamidopyrimidines in distilled water.

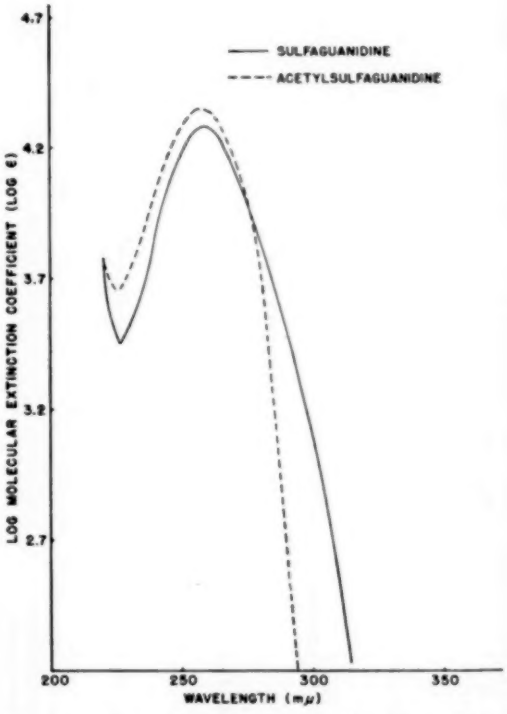


Fig. 4.—Absorption spectra of the sulfaguanidines in distilled water.

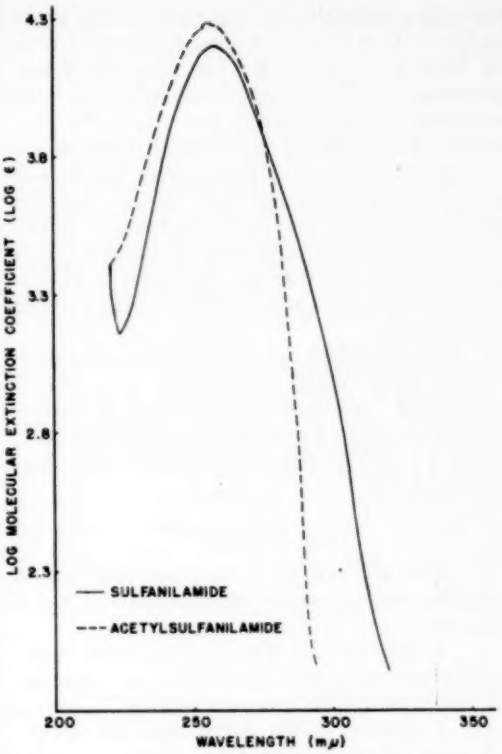


Fig. 5.—Absorption spectra of the sulfanilamides in distilled water.

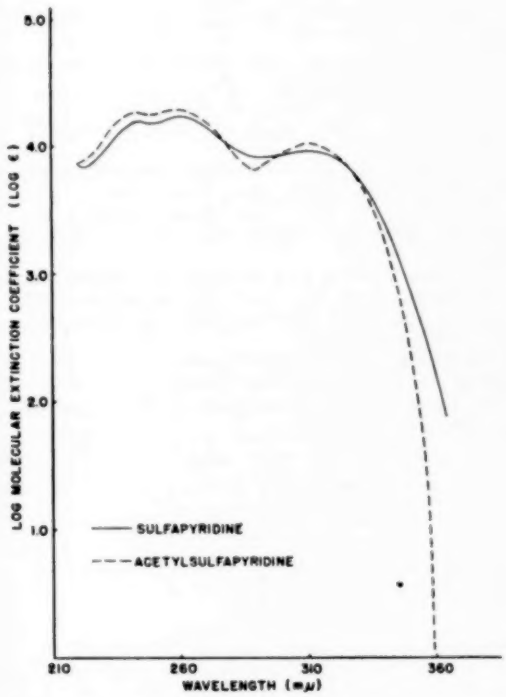


Fig. 6.—Absorption spectra of the sulfapyridines in distilled water.

wave lengths will be nonetheless valuable in differentiating two or more compounds. An inspection of the wave lengths chosen (Table I) will show the reason for their choice as evidenced by the absorption curves given in Figs. 1 to 6. For example, from Fig. 5 it will be noted that the two curves intersect at about λ275 mμ so that the extinction for both sulfanilamide and its acetylated form will be approximately the same at that wave length. On the other hand, acetylsulfanilamide has a slightly higher extinction at its

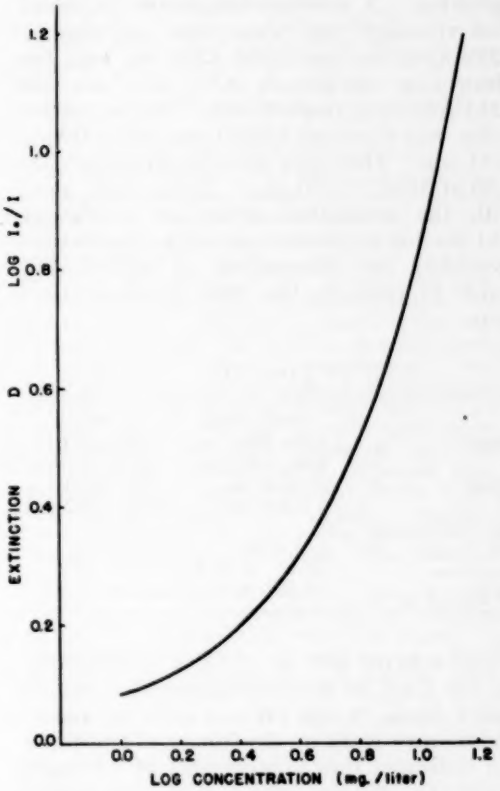


Fig. 7.—Log concentration curve for acetylsulfaguanidine in distilled water at 2580 Å.

absorption maximum (λ255–λ257 mμ) than does sulfanilamide (at λ258 mμ). The absorption ratio of acetylsulfanilamide at λ256/λ275 mμ will then be slightly higher than for sulfanilamide at λ258/λ275 mμ. Within the range of concentrations which follow the Beer-Lambert law the absorption ratios will remain practically constant. In our investigations we have limited ourselves to concentrations of 12.5 mg./L.

If the criteria in Table I are utilized and the unknown sulfonamide under investigation carefully purified, it should be possible to differentiate it from other compounds on the basis of a spectrophotometric examina-

tion. A commercial sample of 2-sulfanilamido-4-methylpyrimidine without previous purification, submitted to us for identification in the laboratory, could not be identified on the basis of spectral absorption alone. This indicates the need for repurification. An illustration of this scheme follows:

An unknown sulfonamide was recrystallized once from 95% methyl cellosolve. The purified compound was dried to constant weight at 100° C. and a solution in distilled water containing 12.5 mg./L. was made up by gently heating in a water bath with constant agitation. A spectrophotometric examination revealed two absorption maxima, at λ238–λ242 mμ and λ263–λ265 mμ and two absorption minima at λ230–λ231 mμ and λ243–λ245 mμ, respectively. The extinction value was 0.910 at λ263.5 mμ and 0.606 at λ241 mμ. This gave an absorption ratio of 1.50 at λ263.5/λ241 mμ. These data, along with the calculated extinction coefficient, and the log molecular extinction coefficient, identified the compound as sulfadiazine. Table II presents the data in summarized form.

TABLE II

	Sulfadiazine	Unknown
λ max.	λ239–λ243 mμ λ263–λ264 mμ	λ238–λ242 mμ λ263–λ265 mμ
λ min.	λ230 mμ λ244 mμ	λ230–λ231 mμ λ243–λ245 mμ
$D\lambda_1/D\lambda_2(\lambda_{263.5}/\lambda_{241\ m\mu})$	1.53	1.50
$\bar{k}_{\lambda_{263.5\ m\mu}}$	72.2 ± 0.4	72.8
$\log \epsilon_{\lambda_{263.5\ m\mu}}$	4.257 ± 0.003	4.261

This scheme may be of value in determining the form in which sulfonamides are excreted from, or are present in body fluids. Preliminary work on this phase of the problem indicates that a good deal of difficulty would be encountered. The absorption maxima of these sulfonamides in urine at *p*H values of 6, 7, 8 and 9 as well as in human ascitic fluid, blood plasma and serum in some cases seem to be slightly shifted, usually to regions of lower wave lengths and are generally more diffuse than in distilled water. One other difficulty is that most of the body fluids studied have a marked absorption in the region necessary for the spectral determination of these sulfonamides. It will, therefore, be necessary to use extraction procedures to eliminate interfering substances.

Some conjugated sulfonamides hydrolyze in aqueous solution, so that the absorption curves reported may not represent undissociated material. To keep hydrolysis at a minimum all measurements were made as rapidly as possible following the preparation of solutions.

2-Sulfanilamido-4,6-dimethylpyrimidine was obtained in the anhydrous form in all subsequent syntheses. The hemihydrate was only obtained once and it has since been shown to slowly change over into the anhydrous form.

CONCLUSIONS

1. The ultraviolet absorption spectra of seventeen sulfonamides in distilled water are presented.
2. A method for the quantitative spectrophotometric determination of sulfonamides in pure solution is described.
3. A scheme is set forth for the differentiation of sulfonamides utilizing pertinent spectrophotometric data.

REFERENCES

(1) Sheppard, R. G., Brattor, A. C., and Blanchard, K. C., *J. Am. Chem. Soc.*, 64 (1942), 2532.
(2) Bergeim, F. H., Coy, N. H., and Lott, W. A., *Ibid.*, 62 (1940), 1873.
(3) Scudi, J. V., *Science*, 91 (1940), 486.
(4) Elvidge, W. F., *Quart. J. Pharm. Pharmacol.*, 14 (1941), 134.
(5) Brode, W. R., "Chemical Spectroscopy," John Wiley & Sons, Inc., New York, 1939.
(6) Fisher, R. A., "Statistical Methods for Research Workers," Oliver and Boyd, London, 1928.
(7) Munch, J. C., THIS JOURNAL, 27 (1938), 404.
(8) Gibb, T. R. P., Jr., "Optical Methods of Chemical Analysis," McGraw-Hill Book Company, Inc., New York, 1942.
(9) Holmes, W. C., and Scanlan, J. T., *U. S. Dept. of Agr., Tech. Bull.*, 310 (1932).
(10) Cary, H. H., and Beckman, A. O., *J. Optical Soc. Am.*, 31 (1941), 682.

Studies in the Pharmacology of Mercury. II. Distribution of Mercury in Mice Following Administration of Hydrophilic Mercury and Calomel Ointments*

By Thomas H. Maren, Jeanne A. Epstein and Wilfred C. Hand†

The general purpose of these studies has been to provide a background of information on the absorption and fate of mercury preparations which had been designed for percutaneous application. In the first paper of this series (1) the type of water-soluble vehicle used was discussed, and the relative toxicities of mercury and calomel, as well as their degree of percutaneous absorption, were evaluated. This work indicated that calomel is more toxic than metallic mercury, and in this respect agrees with Schamberg, Kolmer and Raiziss (2), and Lumholt (3) who observed that calomel is more rapidly absorbed from the site of intramuscular injections than is mercury, and causes correspondingly greater pathological change. We further showed that mercury penetrated the intact skin of rabbits rapidly, and that this penetration is very much greater than that of calomel.

However, we had no further information on the fate in the body of these mercurials, expressed in terms of daily assimilation of the drugs into the internal organs. In order to evaluate the results of percutaneous absorption experiments using these drugs, it appeared useful to investigate their distribution in the body. This is the subject of the present paper, and it forms in part a basis on which the experimental results of our first paper (1) rests. By using subcutaneous injection we remove the experimental complications that the skin barrier causes in administration to small animals and are able to establish more direct pharmacological evidence concerning the fate of calomel and mercury in the body.

It was assumed that the characteristics of the drugs are not appreciably changed because of the differences between subcutaneous and percutaneous application. The pharmacological effects of these drugs depend upon physical and chemical characteristics which should not be qualitatively altered during passage through the skin.

EXPERIMENTAL

Experiment A.—As we intended to treat a large number of animals with a single dose of drug and to sacrifice these animals at intervals over a long period of time, mice were used throughout. The preparations used were of the water-soluble type, already described (1), and had the following formulas:

Formula I		Formula II	
Ingredients	Per Cent	Ingredients	Per Cent
Mercury	1	Calomel	1.17
Cetyl alcohol	9	Cetyl alcohol	8.83
Duponol ME	0.5	Duponol ME	0.5
Glycerin	5	Glycerin	5
Aerosol OT	1	Aerosol OT	1
Water	83.5	Water	83.5

The mice weighed from 18 to 22 Gm., and 0.014 cc./Gm. body weight of the above formulas was injected subcutaneously on the dorsal side, 42 mice receiving I and the same number receiving II. For a human weighing 70 Kg. this would be equivalent to administration of 10 Gm. of mercury. The mice were housed in animal jars, six to each container, and fed on a standard diet of Rockland pellets, with a continuous, adequate supply of water. Three mice from each group were sacrificed on alternate weekdays during the course of the experiment. Liver, kidney, spleen, brain and blood of these animals were pooled and analyzed for mercury content by a modification of the colorimetric dithizone method. This method is described in a separate paper (4). Blank determinations were made on normal mice, and a slight correction was applied to the figures given. Tables I and II give results in terms of γ Hg/Gm. organ, for the liver and kidney. Data on the spleen, brain and blood are given below:

Spleen: All spleen analyses were negative except that of the first day following the calomel injection, when a level of 6.6 γ Hg/Gm. organ was found.

Brain: Small amounts of mercury (4 γ/Gm.) were found in the brain on the fifth and the eighth day following calomel injection. Other findings were negative except for occasional traces of the drug. Following mercury injection, occasional traces were observed also.

Blood: From two to eight days after calomel injection, a level of 1–2 γ Hg/cc. was observed in the blood. From three to ten days following mercury injection levels ranged from 3 to 16 γ Hg/cc. Thereafter no mercury, or only slight traces, were observed in the blood. The higher level following mercury injection is corroborated by Experiment B below.

The sensitivity of the analytical method is such that 1 γ of mercury may be detected. When the organs and blood of these mice were pooled and analyzed collectively, the limiting figures were approximately 1 microgram of mercury in 1.5 Gm. liver, 0.5 Gm. kidney, 0.1 Gm. spleen, 1 Gm. brain, and in 3 cc. blood.

Sections of liver and kidney from one animal in each group of three were set aside in Bouin's solution for histological examination. The right-hand columns of Tables I and II summarize these observations. The findings confirm those of Kolmer and Lucke (5) and are in general typical of the pathological changes accompanying mercury intoxication.

* Received Oct. 1, 1943, from Wallace Laboratories, Inc., New Brunswick, N. J.

† The authors are indebted to Mrs. B. B. Edwards for the histological data, and to Miss Blanche Kohut for general technical assistance.

TABLE I.—ANALYSES AND PATHOLOGICAL CHANGES OF ORGANS OF MICE FOLLOWING SUBCUTANEOUS INJECTION OF 0.014 CC. 1% MERCURY EMULSION PER GRAM BODY WEIGHT

Days	γ Hg/Gm. Organ[c] Kidney	Liver	Pathological Changes Kidney	Liver
1	3.4	1.2	Normal	Normal
3	34	2	Normal	Normal
5	54	5.6	Progressive tubular degeneration during this period with congestion of glomeruli	Moderate leucocytic infiltration about interlobular veins
8	142	2		
10[a]	160	3		
12	100	3		
15	100	3		
17	75	1	Interstitial infiltration and tubular nephrosis	Increasingly marked perivascular infiltration. Dilation of interlobular veins
19	89	1		
22	64	4		
25	116	7		
32	42	Trace	Scar tissue replacing damaged tissues	
37[b]	Trace	Trace		
Average for 32 days at 12 intervals	83	5		

[a] Injection site analyzed. Large amounts of mercury present.
[b] Injection site free of mercury.
[c] Each figure is based on pooled organs of three mice.

TABLE II.—ANALYSES AND PATHOLOGICAL CHANGES OF ORGANS OF MICE FOLLOWING SUBCUTANEOUS INJECTION OF 0.014 CC. 1.17% CALOMEL EMULSION PER GRAM BODY WEIGHT

Days	γ Hg/Gm. Organ[b] Kidney	Liver	Pathological Changes Kidney	Liver
2	83	8	Degeneration of convoluted tubules	Progressively marked parenchymal infiltration
5	133	30		
7[a]	125	79		
8	180	2		
10	111	4	Progressive interstitial infiltration with glomerular tubular damage	
12	67	4		
14	125	20		
16	120	8		Enlargement of central vein
19	150	6		
21	240	25		Distortion of trabeculae and dense parenchymal infiltration
24	20	2	Continued degeneration with appearance of scar tissue	
28	30	2		
Average for 28 days at 12 intervals	115	115		

[a] These animals were found dead on the seventh day.
[b] Each figure is based on pooled organs of three mice.

It was considered of interest to find in what valency form the mercury was deposited in the tissues. Frozen sections of the liver and kidneys were accordingly subjected to differential reductions by means of a technique to be described in a separate publication (6). In both the calomel and mercury series, the preponderance of the metal was present in divalent form. We do not consider that we have enough information at this time to comment on the pharmacological significance of this finding.

Discussion and Observation (A).—From Tables I and II assimilation of mercury in the kidney and liver may be studied. On the first day after injection of II (calomel) the kidney concentration is higher than after five days following injection of I (mercury). It is clear that the absorption by the blood and deposition in the kidney are considerably more rapid following calomel injection. With this drug a high kidney level is attained within a day, and maintained for three weeks. Table II shows a peak at twenty-one days. Subsequently the level falls off rapidly, and the kidney is usually free of mercury by the thirtieth or thirty-first day. The average level for this period is 115 γ Hg/Gm. kidney.

In experiments following injection of mercury, the kidney concentration rises rather slowly, and remains at a high level from the seventh to the twenty-fifth day. During this period the kidney concentration varied considerably, probably depending on the balance between absorption from the injection site and excretion. Mercury could be detected at the injection site until the thirty-seventh day, at which time no metal could be found either at the injection site or in the internal organs. In these mice (thirty-seventh day) all the mercury had been absorbed from the subcutaneous depot, and excreted. Overall, the average level corresponding to that recorded above for the case of calomel (the first twelve sets of analyses) was 83 γ Hg/Gm. kidney.

No qualitative differences between calomel and mercury in respect to deposition in the kidney and ultimate excretion were noted. Following calomel injection, high levels are more quickly reached, the maximum values are greater, and elimination is more rapid. Overall, average values are 29% higher in the case of calomel. The general pattern of kidney concentration after the fifth day, however, is similar for both drugs. Each shows a rather

TABLE III

Mouse No.[a]	Weight in Gm. 1st Day	2nd Day	3rd Day	4th Day	5th Day
1	14.3	14.2	14.6	15.1	15.0
2	11.6	11.0	10.3	12.0	11.7
3	10.3	10.3	Dead	..	..
4	12.3	14.7	13.8	13.5	12.4
5	16.6	18.1	18.2	18.0	18.4
6	15.0	16.3	16.6	17.1	17.1
7	15.4	14.8	15.6	16.1	14.5
8	15.0	14.9	15.2	15.1	14.7
9	15.7	15.3	13.4	15.2	13.5
10	12.8	13.7	13.1	11.8	11.5
11[b]	13.9	14.0	13.9	14.3	13.8
12	13.1	12.9	14.6	13.5	13.7
13	14.3	13.7	14.4	14.6	15.3
14	12.0	13.4	12.4	13.0	12.4
15	15.2	17.2	16.2	16.4	16.5
16	13.7	13.5	13.6	12.8	11.0
17	17.3	18.8	18.2	17.0	16.8
18	13.0	14.0	12.9	12.4	11.5
19	13.0	14.1	13.4	13.4	13.3
20	14.5	16.2	15.5	14.8	14.8

[a] Mice 1–10 received mercury (I).
[b] 11–20 received calomel (II).

TABLE IV.—MERCURY CONTENTS OF KIDNEY AND LIVER FIVE DAYS AFTER INJECTION OF 0.014 CC./GM. BODY WEIGHT OF CREAM I (Hg)

Mouse No.[a]	γ Hg/Gm. Organ Kidney	Liver
1	60	6
2	37	8.1
3	Died 3rd day	..
4	47	3
5	47	2.7
6	135	8.3
7	39	3
8	52	6
9	107	10.1
10	36	3.8
Average	62	5.6
Standard deviation	33	2.7

[a] Mice individually housed and collared.

wide fluctuation in levels. On the average, however, the concentration following mercury injection is 83 γ Hg/Gm. kidney, whereas for calomel it is 115 γ Hg/Gm. kidney.

Tables I and II also show liver concentration for the same period. Here there is a marked difference between the behavior of the two drugs. Following calomel injection (Table II) the liver has a pronounced tendency to assimilate mercury, although in a most irregular fashion. On the seventh day, three mice of this group died, and these exhibited the highest mercury concentration in the liver encountered in these experiments. These were the only mice in either group that died. Following mercury injection (Table I) there is only a slight storage of metal in the liver. Average values for liver concentration over a four-week period following injection of the mercury preparation were only 30% of the equivalent values following calomel injections.

The striking differences between the two drugs as concerns their relative tendencies to accumulate in the liver may possibly explain the difference in toxicity of calomel and mercury. It is significant that both increased liver absorption and higher toxicity are characteristic of calomel.

No symptoms of intoxication were observed at any time during the course of the experiment, despite the very high concentration of metal in the kidney, and the corresponding pathological changes. These are recorded in Tables I and II.

Summary of Pathological Changes.—The mercury-treated animals showed typical progressive mercurial tubular nephrosis which progressed to the beginning of scar formation during the course of the experiment. These changes involved a limited number of glomeruli. The liver in these animals showed little if any serious pathological changes. There was occasional congestion with cellular infiltration; the latter was observed chiefly about the interlobular veins.

In the calomel-injected group the kidneys showed a marked diffuse glomerulonephritis which progressed through the usual acute and degenerative stages to the point of scar tissue formation; typical mercurial tubular nephrosis was marked.

The liver showed more damage than occurred in the mercury-treated animals. Diffuse parenchymal cellular infiltrations with distortion of the trabeculae and some degeneration of the cuboidal cells were the chief changes noted.

The calomel-treated animals showed more prompt and striking changes in the organs studied than did the animals injected with mercury.

Following the injection of mercury, a soft raised area developed at the site. This persisted until the eighth day, when the outer edges of the area became hard and necrotic. On the ninth and the tenth day, this necrotic skin disintegrated leaving a perforating ulcer. This persisted with little change until the twenty-fifth day, when the ulcer began to heal and the skin resumed its normal appearance. By the thirty-fifth day the ulcer had entirely healed. At the end of the experiment, the surviving mice appeared entirely normal. Following the injection of calomel, the necrosis began several days earlier, and the areas involved were larger. The local damage was considerably greater and the time of healing was several days longer.

Experiment B.—As indicated by Tables I and II, the daily deposition of mercury in the kidney and liver is not uniformly progressive, although general trends seem clearly indicated. The particular concentrations observed may fluctuate because of the chance balance between absorption and excretion, which apparently varies among the animals. By using three mice for each analysis as in the above case this variation tends to average out. It seemed desirable to establish the normal variation in individual mice, and more specifically in application to Experiment A, the normal variation which we could expect from groups of three. In this experiment the mice were collared and individually housed. Ten mice were given the same dose of mercury as given in Experiment A, and ten received a similar dose of calomel (0.014 cc./Gm. body weight). Each mouse was weighed daily (Table III). They were fed on a standard diet of Rockland pellets with a continuous supply of water. On the fifth day they were sacrificed, and liver, kidney, spleen, brain and blood were taken from each of the 20 mice. Table IV records levels of mercury in the kidney and liver of the mice receiving mercury emulsion. Table V gives the same data for the ten mice that received calomel. For the spleen, brain and blood, our earlier findings had indicated such small amounts of mercury to be present that we decided to pool the organs of mice 1–5, 6–10, 11–15 and 16–20 for four group analyses. Thus we had two sets of pooled organs for the calomel- and two for the mercury-injected mice. Results are as follows:

Spleen: Each of the four samples showed a trace of mercury. In no case were there more than 2

γ/Gm. spleen. This agrees with our earlier data.

Brain: The animals receiving mercury showed no metal in the brain in one case, and a slight trace in the other set of pooled organs. Following calomel injection, however, mice in group 11–15 had 3 γ/Gm. and group 16–20 had 2 γ/Gm. mercury present. This also agrees with Experiment A, in showing that, after calomel injection, mercury is found in the brain in larger quantities than it is following injection of metallic mercury.

Blood: In the two sets of pooled blood from the mice receiving mercury, levels were 8.3 and 7.5 γ Hg/cc. blood. From the mice receiving calomel, the two sets of pooled blood had levels of 2.5 and 1 γ Hg/cc. This also agrees with the data of Experiment A.

TABLE V.—MERCURY CONTENTS OF KIDNEY AND LIVER FIVE DAYS AFTER INJECTION OF 0.014 CC./GM. BODY WEIGHT OF CREAM II (CALOMEL)

Mouse No.[a]	γ Hg/Gm. Organ Kidney	Liver
11	131	20
12	102	17
13	91	15
14	54	17[b]
15	124	20
16	93	25
17	96	18[b]
18	87	20
19	78	23
20	85	27
Average	94	20.2
Standard deviation	21.3	3.6

[a] Mice individually housed and collared.
[b] Yellow spots in liver. Histological examination revealed acute atrophy.

Discussion and Observation (B).—From Table IV it is clear that there is a wide variation from one mouse to another as respects the mercury levels of both kidney and liver following a single injection of metallic mercury. For the kidney, after five days the range is from 37 to 135 γ/Gm. and the standard deviation is approximately half the average or mean value of the observations. For the liver the range is of the same order, and the standard deviation again is about half of the average value. The average five-day values for kidney and liver, however, agree remarkably well with the figures of Experiment A given in Table I, which represents a single analysis of the pooled organs of three animals. Experiment B gives 62 and 5.6 for kidney and liver, respectively, and Experiment A gives 54 and 5.6 for the same period following equivalent injections.

From Table V, we may observe that, following injection of calomel, individual variation is not so great as it is following mercury injection. For the kidney, half of the figures lie in the narrow range 85–96 γ Hg/Gm., and the standard deviation is comparatively low. For the liver the entire range is 15–27 γ Hg/Gm. and the standard deviation is proportionately lower than that of any other series.

These observations on normal deviation are applicable only for the particular period of time studied, in this case five days. Table I shows, for example, that in the analyses done before and after the fifth day (third and eighth) there is an enormous change in the kidney level (34–142 γ Hg/Gm.) because it is being progressively built up.

The individual variation taken at any point during this rather steep rise will naturally be greater than that taken during a phase where the change is not so great. This is the case of calomel-treated mice for the same general period of time (second to seventh day) where the kidney levels of mercury were 83, 133 and 125 γ/Gm. organ. This range is less than one-third that of the levels of mercury-treated mice for the same approximate period. Therefore, the considerable difference between the standard deviation of Tables IV and V indicates a real difference in pharmacological phenomena in the two series at the five-day period. In Table IV (mercury administration) this time interval is one where there is considerable change in the direction of mounting levels; for Table V (calomel) at the five-day period the general peak has been reached, and the kidney level is not changing sharply in either direction. Therefore, Table V may be expected to show less standard deviation than Table IV, and we find this to be the case.

TABLE VI.—MERCURY CONTENTS OF KIDNEY AND LIVER 11 DAYS AFTER INJECTION OF 0.014 CC./GM. BODY WEIGHT OF CREAM I (Hg) AND CREAM II (CALOMEL)

Mercury injected—Sacrificed 11th day			Calomel injected—Sacrificed 11th day		
	γ Hg/Gm. Organ			γ Hg/Gm. Organ	
Mouse No.	Kidney	Liver	Mouse No.	Kidney	Liver
21	84	2.5	27	115	6
22	70	6	28	160	7.5
23	32	2.5	29	61	20
24	42	5	30	49	10
25	81	10	31	56	34
26	73	5.3	32	190	9
Average	64	5.2	Average	104	14.4
Standard deviation	18.5	2.5	Standard deviation	56	10

It seemed pertinent to inquire whether the above considerations would hold in the reverse case; *i. e.*, one where the calomel-treated animals were undergoing greater change than the mercury-treated mice at a given time, as shown by Tables I and II. Will individual variation at such a period be greater in the calomel group than in the mercury group? Now we may show from Tables I and II that from the eighth to the fifteenth day there is a much larger variation for calomel-treated mice than for the mercury-injected animals. These changes are not systematic but it is true for the entire period after the eighth day, for mercury storage of both kidney and liver. The eleventh day was selected as an interval on which time we measured *individual variation* in series of calomel- and mercury-injected mice. Six mice were given subcutaneous doses of 0.014 cc. of 1% mercury emulsion/Gm. body weight and six were given equivalent doses of calomel. These were housed and fed according to the description given above, and were sacrificed on the eleventh day. Table VI indicates that *individual variation*, at this time, is substantially greater in the case of calomel.

It is evident that the ranges of individual variation in response to the two drugs differ depending on the length of the experiment. This is strongly suggestive of pharmacological difference between calomel and mercury.

Role of Licking in These Experiments.—The close correlation between the five-day average reported in Table IV, where the animals were collared and individually housed, and that of Table I, where they were living together, indicates that licking of the injection site, even if it takes place, does not result in significant changes in the quantities of drug absorbed.

Statistical Error of Experiment A.—The wide

variation reported in Experiment B for groups of ten animals indicates that figures of Experiment A are to be regarded as those falling within a range, encompassed in general by the standard deviation as reported. The figures of Tables IV–VI and the above observations show further that, rigorously applied, the standard deviation itself depends on the particular pharmacological status at a specific given time. At five days after injection of mercury, for example, the standard deviation is different from that five days after calomel injection. Again at the eleven-day period the range of deviation is entirely different.

Practically, however, despite the wide range involved, the reported values of Tables I and II are not subject to the entire variation of the standard deviation, because three animals were used for each figure. However, it is reasonable to ask with what degree of reliability we can expect three pooled animals to be within an acceptable range of experimental accuracy. The chance that three would be outside the range (three highest, or three lowest, corresponding to the extremes of Tables IV and V) are 1 in 119. It is likely, therefore, that the figures of Experiment A have an accuracy considerably within the limits of the standard deviation.

Pathological Findings.—The gross changes noted at autopsy in the liver and kidneys corresponded to those noted in Experiment A. The higher the concentration of mercury in the parenchyma, the more marked were the changes. The mercury preparation caused no gross changes in the liver but some swelling of the kidney with loss of cortical demarkation. Calomel caused similar but more severe changes in the kidney and in a few instances acute yellow atrophy in the liver. Routine histological studies were not attempted in this experiment.

SUMMARY

High concentrations of mercury may be built up gradually without permanent disability. This may occur during a course of inunction therapy in syphilis. In such cases kidney levels may perhaps be considerably higher than would be indicated by the figures given by Sollman and Schreiber (7) from autopsies following suicidal ingestion of mercury bichloride. They report the average kidney concentration was 38 γ Hg/Gm., liver concentration 20 γ Hg/Gm., and for the spleen 5 γ Hg/Gm. Not only is the kidney concentration much less in the acute intoxications described by them than in the chronic experiments reported here, but the distribution is entirely different. As indicated in Table I, following administration of metallic mercury, the increases in concentration of the metal in the internal organs are almost entirely confined to the kidney. Chronic tolerance for mercury is apparently higher than acute tolerance, and the distribution of metal in the former case tends to be limited to excretory organs.

Following the ingestion of calomel, as indicated by Table II, a high mercury concentration is rapidly attained in the kidney, and a considerable amount of metal assimilated in the liver and spleen. After the second day none is found in the spleen, but the kidney continues to have a concentration of mercury varying irregularly between 67 and 240 γ/Gm., until the twenty-first day, when it begins to drop off. The liver reaches a concentration of 79 γ Hg/Gm. and also varies irregularly until the ultimate decline after the twenty-first day. The distribution is clearly different from that of metallic mercury, and in the greater concentrations of metal in liver and spleen, is more nearly like the distribution reported for mercury bichloride. Animals receiving calomel also had considerably greater amounts of mercury in the brain than those that received metallic mercury. It is possible that calomel is more toxic than mercury because the former is more soluble in the body fluids and because it tends to be deposited in the liver.

Blood concentrations were consistently greater in mice that received metallic mercury. Mercury levels in the kidney are a fairly reliable index of the relative order of chronic absorption in the case of metallic mercury. However, with calomel, and perhaps to a greater degree in the case of the more soluble mercurials, liver analyses are also necessary.

The average amount of metal in the kidney is greater following calomel injection, in the approximate ratio of three to two. In the liver, the ratio of metal following calomel injection to that following mercury injection is four to one.

Thirty-five days after a single subcutaneous injection of mercury or of calomel into mice, all the material has disappeared from the injection site and has been eliminated from the internal organs. Damage at the dorsal injection site has healed and the animals appeared normal. No significant weight losses or other signs of intoxication were observed despite the high concentrations found in the liver and kidney.

REFERENCES

(1) Maren, T. H., and Edwards, B. B., THIS JOURNAL, 32 (1943), 255.

(2) Schamberg, J. F., Kolmer, J. A., and Raiziss, G. W., *J. Cutan. Dis.*, 33 (1915), 819.

(3) Lumholt, S., *Biochem. J.*, 18 (1924), 693.

(4) Maren, T. H., *J. Lab. Clin. Med.*, 28 (1943), 1511.

(5) Kolmer, J. A., and Lucke, B., *Arch. Dermatol. & Syphilol.*, 3 (1921), 531.

(6) Hand, W. C., Edwards, B. B., and Caley, E. R., *J. Lab. Chem. Med.*, 28 (1943), 1835.

(7) Sollman, T., and Schreiber, M., *Arch. Internal Med.*, 57 (1936), 46.

Note on "Determination of Ephedrine by Kjeldahl Distillation"*

By Llewellyn H. Welsh

In a recent publication in THIS JOURNAL (1), a method was described for the determination of ephedrine in ephedrine salts. In the method, the sample is refluxed for one and one-half hours with approximately 14% hydrochloric acid, made alkaline with sodium hydroxide, and the "nitrogen distilled as ammonia" into standard acid. The conversion of an amine to ammonia by such treatment seemed so unusual that the procedure was investigated. The results of the work demonstrate that the process involves nothing more than the distillation of ephedrine with steam.

EXPERIMENTAL

A sample of Mallinckrodt ephedrine sulfate, U. S. P. XI (0.3006 Gm.), was subjected to the procedure of Hilty (1). The distillate consumed 13.79 cc. of 0.1 *N* sulfuric acid, equivalent to 0.2955 Gm. of ephedrine sulfate or 0.2781 Gm. of the hydrochloride. The titrated solution was evaporated to dryness on the steam bath, transferred to a separatory funnel with 15 cc. of water, one drop of 10% hydrochloric acid added, and the methyl red indicator extracted with two 2-cc. portions of chloroform. The solution was made alkaline with sodium hydroxide, and extracted with six 20-cc. portions of benzene. The combined extract was washed with 3 cc. of water which was, in turn, washed with 5 cc. of benzene. After filtering the combined benzene extract and wash through cotton, a slight excess of an ethereal solution of hydrogen chloride was added, and the resulting suspension of crystals was evaporated to dryness and dried at 105° C. The residue so obtained was identified by its optical crystallographic properties[1] and by mixed melting point determinations as *l*-ephedrine hydrochloride. It amounted to 0.2741 Gm., or 98.6% of the weight calculated from the titration.

* Received Feb. 27, 1944, from the U. S. Food and Drug Administration, Washington, D. C.

[1] By George L. Keenan, Microanalytical Division, U. S. Food and Drug Administration.

SUMMARY

The determination of ephedrine by a recently described method has been shown to be based on the volatility of ephedrine with steam rather than on its conversion to ammonia.

REFERENCE

(1) Hilty, W. W., THIS JOURNAL, 33 (1944), 28.

WHO MAKES IT?

The National Registry of Rare Chemicals, Armour Research Foundation, 33rd, Federal and Dearborn Streets, Chicago, Ill., seeks information on sources of supply for the following chemicals:

N-Chloro-*N*-benzoyl 2,4-dichloroaniline (Antiverm)
1,2,3,4-Tetrahydroxy benzene (Apionol)
N-Benzyl diphenylamine (10 lbs.)
β-Hydroxy naphthoic anilide (Brenthol AS) (1 lb.)
Benzyl trimethyl ammonium hydroxide
Mono tert butyl phosphite
Cetyl trimethyl ammonium bromide
Cytosine (10 Gm.)
Di-α-naphthylamine
dl-Camphenilone
Di-lauryl phosphate
2-Mercapto-1,3,4-thiadiazole
Kerasin
Phrenosin
Vitellin
Sphingomyelin